G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b)(6); (b)(3):7 U.S.C. § 8401			PhD	Project Lead	(b)(4); (b)(6)				15,580.00	4,090.00	19,670.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **19,670.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			15,595.00	2,933.00	18,528.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Sr. Scientists (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401				40,051.00	11,843.00	51,894.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**70,422.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**90,092.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	46,408.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Histology Core	5,000.00
9. Service Contracts & Maintenance	4,500.00
Total Other Direct Costs	**55,908.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**150,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.0	150,000.00	78,000.00
		Total Indirect Costs	**78,000.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Darryl Mayes 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**228,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Proj 3 (b)(6); (b)(3):7 UNCBudget Justification.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel

(b)(6); (b)(3):7 U.S.C. § 8401 **Co-Investigator** (b)(4) Months (b)(4) (b)(6); (b)(3):7 U.S.C. § 8401 has expertise in viral pathogenesis and viral immunology, and has worked with models of alphavirus pathogenesis, including CHIKV and VEEV for fifteen years. (b)(6); (b)(3):7 will be responsible for the overseeing the testing of candidate therapeutics for their ability to protect mice from acute CHIKV induced arthritis or VEE-induced viral encephalitis. (b)(6); (b)(3):7 will work in close collaboration with Drs. DeFilippis and Streblow, as well as the other research project leaders to set priorities for which drugs should be tested within the CHIKV or VEE models and to identify promising candidates that show promising in vivo potential so that those candidates can be taken forward for further optimization.

(b)(6); (b)(3):7 U.S.C. § 8401 **Investigator** (b)(4) Months (b)(4) . (b)(6); (b)(3):7 U.S.C. § 8401 of experience working with both CHIKV and VEE in mouse models and will work in close coordination with (b)(6); (b)(3):7 U.S.C. § 8401 to test candidate therapies for their ability to protection from CHIKV or VEE-induced disease. (b)(6); (b)(3):7 will administer therapeutics, perform CHIKV and VEE infections, and will monitor infected animals for disease signs and collect tissues to assess viral loads and virus-induced pathology. (b)(6); (b)(3):7 also directs the day to day operations of the BSL-3 facility where all work with CHIKV and VEE is conducted and will oversee the proper training and compliance of all individuals working within the BSL-3 facility.

(b)(6); (b)(3):7 U.S.C. § 8401 **nvestigator** (b)(4) Months, (b)(4) . (b)(6); (b)(3):7 U.S.C. § 8401 has approximately 10 years of experience working with VEE and CHIKV infected mice under BSL-3 conditions. (b)(6); (b)(3):7 U.S.C. will be responsible for coordinating in vivo mouse studies and will be involved in the administration of candidate therapies and viral challenge studies. (b)(6); (b)(3):7 will also assist in collection of data to assess the impact of therapeutics on viral loads, virus-induced disease, and virus induced pathology within joint (CHIKV) and the central nervous system (VEE).

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) Months (b)(4) (b)(6); (b)(3):7 U.S.C. § 8401 has experience working with alphaviruses and alphavirus molecular clones. (b)(6); (b)(3):7 will be responsible for testing candidate compounds for antiviral activity against CHIKV and VEE, determining what stage in the viral replication cycle the inhibitory compounds are acting, and determining whether resistance mutants arise against the compounds.

Fringe Benefits: Faculty/Staff: 22.741% Social Security and Retirement; $5,471FTE Health Insurance. Postdoctoral fellow benefits: 8.990% for FICA and other fees plus $4,373 fixed premium for health insurance, prorated to effort.

SUPPLIES

The evaluation of candidate therapies against either CHIKV or VEE requires the assessment of viral loads, evaluation of inflammatory cell infiltration and pathology within these tissues. Therefore funds are requested to cover the costs of tissue culture consumables (plastic ware, media, serum) required for the assessment of viral loads within the joints or CNS. We will also need to generate viral stocks, as well as generate infectious clones containing potential escape mutants and funds are requested to cover the costs of the molecular biology supplies needed for those purposes. We are also requesting funds to cover the cost of purchasing adult C57Bl/6 mice, which will be used for testing candidate therapies against both CHIKV and VEE, as well as funds to cover the cost of supplies needed to house these animals within our BSL-3 laboratory. Lastly, since some assays will need to be performed under BSL-3 conditions, funds are requested to cover the cost of personal protective gears, such as gloves, tyvek suits, and PAPRs.

TRAVEL

Funds are requested for the Project Leader, and 2 investigators to attend 1 scientific meeting to present findings and interact with other scientists in the field and to attend programmatic meetings.

OTHER EXPENSES

Equipment service contracts ($4,500): Several instruments in the (b)(6); (b)(3):7 Laboratory that will be used in these studies (4deg centrifuge, CO2-incubators, microscopes) require service contracts for regular maintenance and repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology Costs ($5,000) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

Federal F&A Cost Rate: In accordance to an agreement between DHHS and UNC dated May 16, 2012, the indirect cost rate is 52% of modified total direct costs.

A. COMPONENT COVER PAGE

Project Title: Project 3.3 Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses

Component Project Lead Information:

MORRISON, THOMAS E

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphaviruses are broadly comprised of geographically derived clades: New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinct pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean and Caribbean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America. Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease. Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication, demonstrating that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease. Since the target of this class of inhibitors, namely RNA- dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds that impact these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 3), Coronaviruses (Project 2), and Influenza (Project 4). In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating in vitro replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV. Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique molecular libraries. Our goal is the identification of lead molecules for further in vivo evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents. In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.
Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity. Initial use of this assay has already identified several compounds with antiviral activity against VEEV. Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.
Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the Alphaviruses VEEV and CHIKV. Following these initial screens, “hits” will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC50 , CC50 , and selective indices.

Aim 2: Validate and characterize antiviral activity and off-target effects.
Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to efficacy and mechanism of action.
Strategy: We will use a variety of secondary assays to identify: 1) breadth of anti-Alphavirus activity (test multiple Alphavirus species); 2) cell type-specificity (biologically relevant cells); 3) targets of antiviral compounds; and 4) ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of in vivo efficacy of lead compounds.
Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus. Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.
Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Project 3 Morrison B2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

The plans for Year 3 in Project 3 can be found under the information for Project 3.1.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

The accomplishments for Yr2 in Project 3 can be found under the information for Project 3.1.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Thomas		Morrison	PhD	Project Lead	(b)(4); (b)(6)				27,281.00	7,639.00	34,920.00
Total Funds Requested for all Senior Key Persons in the attached file													
Additional Senior Key Persons:			File Name:									**Total Senior/Key Person**	**34,920.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	(b)(4)					
1	Graduate Students				14,250.00	0.00	14,250.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Professional research assistant				19,864.00	5,562.00	25,426.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**39,676.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**74,596.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		6,000.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**6,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	29,495.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal expenses	40,000.00
Total Other Direct Costs	**69,495.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**150,091.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified Total Direct Costs	55.5	144,091.00	79,971.00
		Total Indirect Costs	**79,971.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS Arif Karim (214-767-3600)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**230,062.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Project_3_Budget_Justification Morrison Done MW 12.10.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel ($74,596)

Thomas E. Morrison, Ph.D., Co-Investigator (b)(4) **months).** Dr. Morrison has extensive experience and expertise in alphavirus pathogenesis, host immune responses to virus infection, and mouse models. Dr. Morrison will be responsible for the overall administration and direction of the project. He will be responsible for the overseeing studied designed to determine the mechanism of action of select compounds and for the testing of candidate therapeutics for their ability to protect mice from chronic CHIKV infection and disease. He will work in close collaboration with Drs. Heise, DeFilippis and Streblow, as well as the other research project leaders to set priorities for which drugs will be evaluated for mechanism of action and which will be tested within the CHIKV chronic disease model.

David Hawman, Graduate Student in Microbiology (b)(4) **months).** Mr. Hawman will perform mechanism of action studies in cells for select compounds. Mr. Hawman also has experience working with the CHIKV chronic disease model and high-throughput sequencing of CHIKV sequence specific amplicon libraries. He will perform CHIKV inoculations of mice, administer therapeutics, perform animal necropsies, and process tissues for quantification of CHIKV RNA and for the assessment of tissue pathology.

Nick May, Professional Research Assistant (b)(4) **months).** Mr. May will assist Mr. Hawman in testing therapeutics for their ability to protect mice from chronic CHIKV infection and disease. He will perform real time PCR analysis to evaluate viral RNA loads in virally infected tissues in the presence or absence of candidate therapeutics. In addition, Mr. May will perform mechanism of action studies in cells for select compounds.

Other significant contributors

Stephanie Montgomery, Ph.D., D.V.M., North Carolina State University, Raleigh, NC (no funds requested). Dr. Montgomery and Dr. Morrison have an active collaboration related to CHIKV-induced tissue pathology that was an important component of two previous publications. Dr. Montgomery, a veterinary pathologist with a doctorate in alphavirus biology, will provide expert analysis of histopathological changes in murine tissues.

Supplies ($29,495)

The evaluation of candidate therapies for the treatment of chronic CHIKV infection and disease in the mouse model requires stocks of infectious CHIKV. Therefore, we request funds for tissue culture consumables and transcription kits. To evaluate the effects of candidate therapies requires the isolation of RNA from specific tissues and the quantification of CHIKV RNA via qRT-PCR and preparation of tissues for evaluation of histopathologic changes. Therefore funds are requested to cover the costs of consumables such as Trizol, RNA isolation kits, reverse-transcriptase enzyme, and PCR reagents. We will also need to generate H & E stained tissue section for evaluation of histopathologic changes. In addition, we request funds for reagents and fees associated with high-throughput sequencing of any identified resistance mutant that emerge out of the animal experiments. Lastly, since some assays will need to be performed under BSL-3 conditions, funds are requested to cover the cost of personal protective equipment, such as gloves and tyvek suits.

Animals ($40,000)

The CHIKV chronic infection model utilizes three-to-four week old C57BL/6 mice. Therefore, we request funds to cover costs associated with the purchase of breeding pairs of mice, costs associated with maintaining an active breeding colony, and costs associated with housing experimental mice for up to 4-6 months under ABSL3 conditions.

Tuition Remission ($6,000):

Tuition Remission is provided for the Graduate Student.

A. COMPONENT COVER PAGE

Project Title: Project 4.2 Identification and characterization of novel drugs that target the influenza virus polymerase functions

Component Project Lead Information:

(b)(6); (b)(3):7 U.S.C. § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overall goal of this project is to identify new therapies that target influenza virus replication. The emergence of highly pathogenic strains of influenza virus has highlighted the urgent need for new effective treatments. A primary concern with the current drugs used to treat influenza is the development of resistance mutations that negate therapeutic benefit. Antiviral resistance to the M2 ion channel inhibitors (adamantanes) increased sharply in Asia beginning in 2002. Subsequently, by 2005 in the United States, 92% of influenza A (H3N2) isolates had developed high level resistance to this class of drugs. Currently, the CDC recommends that neither amantadine nor rimantadine be used for the treatment or chemoprophylaxis of influenza A in the US. Evidence in the literature suggests that targeting the influenza virus RNA dependent RNA polymerase (RdRp) is a rational approach for antiviral therapy. The RdRp is responsible for a number of functions including 5'cap recognition, endonuclease activity, replication, transcription, and polyadenylation. Recently, cryo-EM reconstitution studies identified branched-RNP structures as putative replication intermediates and suggested a mechanism for viral replication by a second polymerase activity on the RNP template {Moeller, 2012 #383}. The second polymerase activity is believed to be a function of the polymerase complex. Clearly, the RdRp provides multiple functional domains that could be targets for antiviral drug therapy. Previous studies showed that mutations in the conserved regions of PB1 subunit of the polymerase complex produce inactive RNA polymerase {Biswas, 1994 #389}. We hypothesize that the viral escape mutants resulting from drugs targeting the influenza polymerase might produce inactive RdRp that is unable to replicate the viral genome.

The specific aims of the original proposal:

Aim#1. Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block influenza virus replication.

Hypothesis and rationale: Targeting the influenza polymerase activity might prove more effective than targeting the viral glycoproteins. There are multiple functional domains of influenza polymerase that are rational targets for antiviral therapy. Previous studies demonstrated that point mutations of the conserved regions of PB1 produce inactive polymerase. We hypothesize that viral resistance to novel compounds that target the polymerase activity might attenuate or inactivate the viral polymerase. It is also likely that we will identify compounds against the conserved regions of influenza virus polymerase subunits that might be effective against multiple viral strains.
Experimental strategy: We will use established CPE-based assays to screen novel libraries against influenza viruses. We will use this assay to screen small molecule libraries that have not been previously screened for activity against human pathogens. These libraries are composed of highly diversified small molecules that contain novel and original drug-like features with distinct topologies and diverse functionalities.

Aim#2: Characterize the antiviral activity of hit compounds and identify anti-polymerase inhibitors.

Hypothesis and rationale: The CPE-based HTS screening will identify hit compounds that target several stages of the virus life cycle, including multiple functional domains of the influenza RNA polymerase. It is critical to design an experimental strategy that will focus our analysis on the hit compounds that block post-entry steps of viral infection.
Experimental strategy: To identify the hit compounds that target the viral polymerase we will first use a viral entry assay to eliminate hit compounds that target the first step of the infection cycle. Elimination of hit compounds that target the interaction of the virus glycoprotein with the host cell receptor will focus the search for post-entry inhibitors including anti-polymerase. Additionally, we will eliminate hit compounds that induce interferon. Several secondary and tertiary assays will be performed to determine the viral protein target of the hit compounds.

Aim#3: Chemical optimization and determination of the in vivo efficacy of lead compounds.

Hypothesis and Rationale: Our secondary assay characterization is expected to identify multiple compounds that are specifically effective in inhibiting influenza replication. Optimization of the effective scaffolds should generate compounds with greater efficacy, selectivity, and bioavailability.
Experimental strategy: The hit compounds from the HTS will be triaged and progressed as outlined in the Chemistry core. Compounds with the appropriate activity and pharmacokinetic properties will be evaluated using in-house mouse infection models.

There are no changes in either the goals or specific aims as originally submitted.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Project (b)(6); (b)(3):7 U.S.C. § 32.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Project 4 (b)(6); (b)(3):7 32.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

The plans for Year 3 in Project 4 can be found under the information for Project 4.1.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

The accomplishments for Yr2 in Project 4 can be found under the information for Project 4.1.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

The accomplishments for Yr2 in Project 4 can be found under the information for Project 4.1.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b)(6); (b)(3):7 U.S.C. § 8401			PhD	Project Lead	(b)(4); (b)(6)				20,326.00	9,106.00	29,432.00
2.					PhD	Co-Project leader					16,467.00	7,377.00	23,844.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **53,276.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	(b)(4)			44,530.00	19,948.00	64,478.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	2 Biologists, BSL3 Differential Pay (64 hrs x $2)				77,403.00	34,619.00	112,022.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**176,500.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**229,776.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	10,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**10,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	114,701.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. BSL3 facility fees	8,000.00
Total Other Direct Costs	**122,701.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**362,477.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH - Salaries + Benefits	120.0	229,776.00	275,731.00
2. G&A - Total Direct Cost + OH	20.0	638,208.00	127,642.00
3. CFC - Salaries + Benefits	4.6	229,776.00	10,570.00
4. CFC - Total Direct Cost + OH	1.0	638,208.00	637.00
		Total Indirect Costs	**414,580.00**

Cognizant Federal Agency DHHS, Steven Zuraf, 301492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**777,057.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget SR Project 4.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Please wait...

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For more assistance with Adobe Reader visit http://www.adobe.com/go/acrreader.

Grant Number: 5U19AI109680-04
FAIN: U19AI109680

Principal Investigator(s):
Richard J. Whitley, MD

Project Title: Antiviral Drug Discovery and Development Center - Overall

Shaun Pryor
Dir, Ofc of Sponsored Progs
Univ of Alabama at Birmingham
AB 1170
701 20th Street South
Birmingham, AL 352940111

Award e-mailed to: OSP-NGA@mail.ad.uab.edu

Period Of Performance:
Budget Period: 03/01/2017 – 02/28/2018
Project Period: 03/01/2014 – 02/28/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $7,293,471 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF ALABAMA AT BIRMINGHAM in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number U19AI109680. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Devon R. Bumbray-Quarles
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5U19AI109680-04

Award Calculation (U.S. Dollars)

Salaries and Wages	$135,737
Fringe Benefits	$43,214
Personnel Costs (Subtotal)	$178,951
Consultant Services	$12,500
Materials & Supplies	$16,660
Travel	$51,572
Other	$24,931
Subawards/Consortium/Contractual Costs	$6,875,088
Federal Direct Costs	$7,159,702
Federal F&A Costs	$133,769
Approved Budget	$7,293,471
Total Amount of Federal Funds Obligated (Federal Share)	$7,293,471
TOTAL FEDERAL AWARD AMOUNT	$7,293,471
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$7,293,471

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
4	$7,293,471	$7,293,471
5	$7,112,904	$7,112,904

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1636005396A6
Document Number:	UAI109680A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2017

IC	CAN	2017	2018
AI	8026797	$157,657	
AI	8472315	$7,135,814	$7,112,904

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M65B B / **OC**: 414P / **Released**: (b)(6) 03/14/2017
Award Processed: 03/15/2017 12:04:00 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5U19AI109680-04

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5U19AI109680-04

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.

d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) U19AI109680. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award is funded by the following list of institutes. Any papers published under the auspices of this award must cite the funding support of all institutes.

National Institute Of Allergy And Infectious Diseases (NIAID)

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made

publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5U19AI109680-04

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

This award provides supplemental funds in the amount of **$157,657** Total Costs; (**$95,000** Direct Costs, **$62,657** F&A Costs) for the **Zika Supplement/ Administrative Supplement**. These funds provide support for the period **03/01/2017 - 02/28/2018**. These funds are restricted for stated purpose, in request dated **04/01/2016**,from **Richard Whitley and Stephanie May / University of Alabama at Bitmingham**, and may not be rebudgeted or used for any other purpose, without NIAID awarding unit approval.

*****

This award includes funds awarded for subrecipient activity with **Southern Research Institute** in the amount of **$4,225,338** (**$2,221,145**direct costs + **$2,004,193**facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **Oregon Health and Science University** in the amount of **$1,075,293** (**$634,239**direct costs + **$441,054**facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **Vanderbilt University** in the amount of **$404,625** (**$256,092** direct costs + **$148,533** facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **The University of North Carolina at Chapel Hill** in the amount of **$690,644** (**$454,371** direct costs + **$236,273** facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **Washington University** in the amount of **$249,126** (**$163,361** direct costs + **$85,765** facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **University of Colorado at Denver** in the amount of **$230,062** (**$147,950** direct costs + **$82,112** facilities and administrative costs).

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps/HTML5/section_15/15_consortium_agreements.htm.

*****

This award is issued as a Cooperative Agreement, a financial assistance mechanism in which substantial NIH scientific and/or programmatic involvement is anticipated in the performance of the activity. This award is subject to the Terms and Conditions of Award as set forth in Section VI: Award Administrative Information of **RFA AI-12-044**, "**Centers of Excellence for Translational Research (CETR) (U19)**," posted date **11/23/12**, which are hereby incorporated by reference as special terms and conditions of this award. [If applicable please add: These special Terms and Conditions of Award were included on the award notice for the **-01** year issued on **02/12/14**.]

This RFA may be accessed at: http://grants.nih.gov/grants/guide/index.html

*****

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at

http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

*****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.
*****

This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Gregory P. Smith
Email: gsmith@niaid.nih.gov **Phone**: 240-669-2993 **Fax**: 301-493-0597

Program Official: Maureen J. Beanan
Email: beananm@mail.nih.gov **Phone**: 240-292-0999

SPREADSHEET SUMMARY
GRANT NUMBER: 5U19AI109680-04

INSTITUTION: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Budget	Year 4	Year 5
Salaries and Wages	$135,737	$116,955
Fringe Benefits	$43,214	$35,494
Personnel Costs (Subtotal)	$178,951	$152,449
Consultant Services	$12,500	$10,000
Materials & Supplies	$16,660	$22,100
Travel	$51,572	$55,000
Other	$24,931	$30,619
Subawards/Consortium/Contractual Costs	$6,875,088	$6,715,757
TOTAL FEDERAL DC	$7,159,702	$6,985,925
TOTAL FEDERAL F&A	$133,769	$126,979
TOTAL COST	$7,293,471	$7,112,904

Facilities and Administrative Costs	Year 4	Year 5
F&A Cost Rate 1	47%	47%
F&A Cost Base 1	$284,614	$270,168
F&A Costs 1	$133,769	$126,979

A. OVERALL COVER PAGE

Project Title: Antiviral Drug Discovery and Development Center - Overall	
Grant Number: 5U19AI109680-04	**Project/Grant Period:** 03/01/2014 - 02/28/2019
Reporting Period: 03/01/2016 - 02/28/2017	**Requested Budget Period:** 03/01/2017 - 02/28/2018
Report Term Frequency: Annual	**Date Submitted:** 12/19/2016
Program Director/Principal Investigator Information: RICHARD J WHITLEY , AB MD **Phone number:** 205-934-5316 **Email:** rwhitley@peds.uab.edu	**Recipient Organization:** UNIVERSITY OF ALABAMA AT BIRMINGHAM UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave South, AB990 BIRMINGHAM, AL 352940001 **DUNS:** 063690705 **EIN:** 1636005396A6 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: SHAUN R PRYOR 701 20th Street South AB 1170 Birmingham, AL 35294 **Phone number:** 2059662395 **Email:** spryor6@uab.edu	**Signing Official:** PATRICK MONROE 1720 2nd Avenue South AB 1170 Birmingham, AL 35294 **Phone number:** 205-934-2146 **Email:** ptmonroe@uab.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The past 15 years have witnessed the emergence and re-emergence of several human viral infections of life threatening proportions, including diseases attributable to SARS coronavirus, highly pathogenic H5N1 influenza, pandemic 2009 influenza, monkeypox imported into the United States (US), West Nile virus (WNV) and dengue. Arguably, no efficacious therapy exists for most of these diseases and resistance is a threat to circulating influenza. Experimental approaches have been applied to each one of these diseases but with varying degrees of success.

The goal of this program is to form the Antiviral Drug Discovery and Development Center (AD3C) and identify compounds working though mechanisms that affect viral RNA replication and, importantly, to develop these leads in a translational manner to new human therapeutics. All four projects in this program are focused on viruses deemed critical to NIAID's focus on Emerging and Re-emerging Infectious Diseases related to biodefense. The projects perform High Throughput Screening utilizing unique compound libraries to identify novel chemical scaffolds with antiviral activity. Importantly, the projects report strong preliminary data that demonstrate the feasibility of performance of proposed mechanistic analysis of inhibitory compounds. In addition, all projects already have existing active compounds that will enter the drug discovery and development pathway at a later stage for evaluation.

The common theme of our application is targeting viral RNA replication. The experimental strategies designed by the four projects will provide a comprehensive analysis of the mechanism of action of the potential hit compounds. For example, it has been known for a long time that there are four consensus sequences that are conserved among the RNA-dependent RNA polymerases encoded by plus, minus and double stranded RNA viruses (1). The novel drug libraries with their diverse functionalities will allow the identification of compounds that might target conserved regions of the polymerase and thus yield broad-spectrum antiviral compounds. Based on the existing data in the literature and the preliminary data generated in the laboratories of the four groups we hypothesize that the development of drugs, which target enzymes such as polymerase and 2'O-methyl-transferase are rational approaches for the treatment of these viral diseases and will be more effective than targeting the surface glycoproteins. Resistance to drugs targeting the glycoproteins has frequently been reported. We hypothesize that viral escape mutants resulting from drugs targeting polymerase will be unfit for RNA replication, based on recent data in the literature. This data demonstrated that the mechanism of activity by the reported T-705 anti-polymerase drug is by inducing lethal mutagenesis in the polymerase protein, resulting in a nonviable virus unfit for replication. In AD3C, we will combine the virus-specific knowledge of leading virologists in the world with the high throughput screening and medicinal chemistry and lead optimization capabilities of Southern Research. The program's general specific aims are thus to:

1.Test viral targets essential to RNA replication in high-throughput-screening assays with unique chemical libraries to establish lead molecules for drug discovery.
2.Validate lead compounds in secondary and tertiary assays to confirm selectivity and mechanism of action as well as assure absence of off-target effects.
3.Probe the effects of lead molecules in representative animal models of targeted diseases and utilize such data to define impact on disease pathogenesis. Medicinal chemistry will optimize leads and further define platforms.

The individual projects all follow the general approach as described above, and are led by Drs. Jay Nelson (OHSU) and Michael Diamond (Washington University) to study compounds active against flaviviruses; Drs. [redacted] (Vanderbilt) and Ralph Baric (UNC – Chapel Hill) to study compounds active against SARS-coronavirus; Drs. Dan Streblow (OHSU) and [redacted] (UNC – Chapel Hill) to study compounds active against alphavirus and Drs. Ghalib Alkhatib, Jim Noah (Southern Research) and Rich Whitley (UAB) to study compounds active against influenza. All projects will extensively utilize the Screening Core and Medicinal Chemistry and Lead Development Core at Southern Research, which, with seven FDA approved drugs, has an outstanding track record of bringing drug discovery programs to clinical reality. All this will be coordinated out of the Administrative Core at UAB, which has extensive experience in drug discovery programs.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Umbrella B2 EAB Report.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Umbrella B4 IDPs.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Year 4 will focus on the further development of tractable molecules that have been identified from different platforms that will continue to emerge from the chemistry efforts. For some of these molecules, we have already initiated and will continue to identify how viruses will develop resistance to these new compounds, and map newly found resistance mutations to identify the compounds' molecular targets. Select compounds will be evaluated for microsomal stability and pharmacokinetic parameters such as plasma half-life, oral bioavailability and bio-distribution, to facilitate therapeutic studies in infection models in rodents.
We will also initiate combination studies when compounds work through different mechanisms of action, to look for synergy and avoidance of resistance development. Finally, progress in the last year should clarify which compounds would be suitable candidates to enter pre-IND enabling studies, going forward.

September 14-15, 2016 Annual Meeting
External Advisory Board Report

Attendees: Dr. Kara Carter, Dr. Fred Hayden, Dr. George Painter, Dr. Pei-Yong Shi, EAB Board members, Dr. Maaike Everts, AD3C co-director and Mary Wyatt Bowers, meeting recorder.

The AD3C External Advisory Board met on September 15, 2016, following the conclusion of the annual meeting, to discuss and evaluate the progress of the four projects and three cores.

Overall comments:

The EAB expressed a favorable impression on how the project and core PIs work together; there is clearly good communication between the groups, and everyone seems to be on the same page, with respect for each other's expertise.

With that said, inevitably, some projects will result in better lead molecules than others, and the EAB strongly suggests that the consortium identifies criteria and timelines for the various medicinal chemistry programs, so that resources at some point can converge on the most productive chemical series for a certain virus (family). This may result in the termination of programs for other viruses. Although these decisions may be unpopular, they will benefit the development of a candidate molecule ready for IND-enabling studies at the end of the 5-year funding period.

The EAB emphasized that at this point investigators should not be focused on developing non-human primate models (some models were presented during the annual meeting) as this is not a focus of the consortium; for programs focused on viral infections in which clinical trials are possible, demonstration of activity in a murine model may be adequate to support further development. If development is under the FDA's Animal Efficacy Rule than a second model can be explored via consultation with FDA after a development candidate has been identified.

Another general suggestion was that when compounds are tested for efficacy in animal models, tissue samples are taken so that tissue drug levels and biodistribution can be determined, to complement pharmacokinetic studies. It is imperitive to determine if distribution of the compound is appropriate for the pathogenic course of the disease. Ultimately, depending on mechanism of activiral action, it may be the Cmax, volume of distribution, or other pharmacokinetic parameter in either plasma or tissues that drives the pharmacodynamic effect, not necessarily half-life in plasma.

The EAB would further like to suggest that when active scaffolds are identified, mechanism of action studies extend beyond identification of resistance mutations. Additional experimental approaches include assays employing thermal stability, the use of affinity labeled compounds, cocrystalization, etc, to clearly show a drug-binding pocket interaction. This might also inform what off-target toxicities need to be looked out

for. The board was pleased to hear that the chemistry core budget includes resources to look at receptor panels, etc.

Although the consortium has been charged with finding broad spectrum agents, the EAB cautions that compounds that seem to work against many if not all viral families, the target may be a host target, with all its associated safety issues. Broad spectrum is certainly a goal, but should be clearly defined as 'two or more', and it is already considered a 'win' even if those two viruses are within the same family, so for example a compound that works against both CHIKV and VEEV. To be looking for compounds that would work across, filo-, flavi, alpha-, corona- and influenza viruses should not be the priority.

Finally, the EAB commented on the high quality of the CPE curves in many of the projects; for the viral load reduction curves, they suggested looking at the inflection point of the curves instead of the 'traditional' IC50 or IC90 numbers, since the Y-axis on these curves is logarithmic. A 'work-around' is to use the exponential of the numbers (so eg 3 for 1000, 4 for 10,000, etc) on the Y-axis, thereby allowing software to calculate the inflection point of these curves. The EAB members predict that those values would more closely align with the numbers obtained from CPE curves.

Overall, the greatest strength of the consortium is knowledge of the biology of the viruses. The EAB was reluctant to identify any weaknesses, but as a point of advice, strongly suggested that the chemistry efforts get concentrated on the most promising projects sooner rather than later. To reiterate an earlier statement, setting up criteria and, importantly, timelines, will be critical in this regard.

Core and Project Specific Comments:

Core A - Admin Core -The administrative core is doing a great job facilitating the communication between all cores and projects; the EAB expressed its admiration for the participating PIs to not let egos get in the way and freely work together.

Core B – Screening Core – as outlined in the original proposal, the efforts in the core are winding down with only a few screens remaining that are wrapping up. EAB members noted again that at this stage in the project, about halfway through, investigators need to have identified lead molecules and be moving forward, with the core supporting SAR determination.

Core C - Medicinal Chemistry and Lead Optimization Core – the EAB said that this is the most critical core at this point, and they suggested that core director Ashish Pathak be made the lead co-PI overall on all projects. Dr. Pathak appears to work well with the teams, particularly Dr. Streblow. The core has established target criteria for all programs, and the EAB suggests strict adherence to these criteria along with timelines, to allow discontinuation of non-performing projects, thus focusing resources on the most productive lines of investigation.

Project 1 – Flaviviruses – EAB members noted that this is an intrinsically difficult virus to work with, and are not surprised at the limited success of the chemistry efforts. The EAB expressed concern for the quality control of secondary assays done at OHSU; they would like to see control compounds run at every testing cycle, and validation of the robustness of the assays employed. There was confusion amongst the EAB members with the presentation of Zika virus data during the annual meeting, and they recommend to keep the focus of the project on dengue and WNV.
Now that the WNV screen is (almost) finished, the EAB is looking forward to the identification of potentially other and additional scaffolds to explore anti flavivirus activities.

Project 2 – Coronavirus – EAB members agreed that this project is doing nice work, in particular with the Gilead compound. It was clear that the team works well together, and everyone is in communication, taking advantage of the expertise of each investigator. Board members suggested the investigators consider looking for different mechanisms of actions among hits coming out of the HTS done at SR. This would allow the exploration of combinations of drugs, and potentially synergy with compounds such as those from Gilead. This would include drugs approved for other indications that have shown activity in animal models and used in treating SARS and MERS patients like lopinavir and type 1 interferons.

Project 3 – Alphaviruses – this project is doing really well and making great progress, particularly with the Chikungunya efforts. Going forward and looking ahead to in vivo experiments, the EAB cautioned that with VEEV in particular, the researchers must confirm that the compounds get into the CNS before investing a significant amount of time and effort in conducting animal efficacy studies. While evaluating compounds and scaffolds for microsomal stability by looking at how much of the original compound is left after certain time points, the EAB also suggests looking for metabolites in vitro, to get a sense of metabolic pathways involved.

Project 4 – Influenza – this remains the least advanced project. EAB members recommended that the investigators get rid of the “laundry list” of assays proposed during the annual meeting and focus on finding compounds with the desired mechanism of action (ie, polymerase inhibitors) as quickly as practically possible. They noted that many of the hits are from the Gilead collection; the chemists at Gilead can probably help narrow down the compounds of interest, by eliminating those for which the target has already been identified. The EAB suggested several names of external experts who could provide advice regarding optimization of a polymerase-based assay.

IDPs:

Please refer to the Project descriptions for Individual Development Plans used by the respective institutions with which the trainees are affiliated.

C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	McCarthy MK, Morrison TE. Chronic chikungunya virus musculoskeletal disease: what are the underlying mechanisms?. Future microbiology. 2016;11(3):331-4. PubMed PMID: 26939523; PubMed Central PMCID: PMC4842256.
Complete	Everts M, Suto MJ, Painter GR, Whitley RJ. Consortia's critical role in developing medical countermeasures for re-emerging viral infections: a USA perspective. Future virology. 2016 March;11(3):187-195. PubMed PMID: 27325914; PubMed Central PMCID: PMC4912138.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Research Material	Limited quantities of most synthetic compounds will be made available to qualified individuals for research purposes once the pertinent data has been published. As stated in the original Resource Sharing Plan for Core C (p. 334 of application), once published, and while compound supplies last, we will make research samples of synthetic compounds available to the scientific community for use as chemical probes and for other biological studies. Moreover, we fully expect all biological and chemical data to be published in scientific manuscripts after appropriate patent protection is in place. At this early stage of chemistry, there is no specific resource sharing to report.

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Component(s)	Country	SS
(b)(6)	Y	Whitley, Richard J.	(b)(6)	(b)(6)	AB,MD	PD/PI	(b)(4); (b)(6)				Admin Core-8274 (Administrative Core - Core A)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401				Non-Student Research Assistant					Project-8276 (Project 2.1 Inhibitors of … Therapeutics)		NA
	N	Collins, Deborah				Technician					Project-8278 (Project 4.1 Identification …ase functions)		NA
	N	Eagar, Jessica			MS	Technician					Project-8278 (Project 4.1 Identification …ase functions)		NA
	Y	Everts, Maaike	(b)(6)	(b)(6)	PHD	Co-Investigator					Admin Core-8274 (Administrative Core - Core A)		NA
	N	Patton, Katherine		(b)(6)	BS	Technician					Project-8282 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	Rice, Terri			BS	Technician					Project-8278 (Project 4.1 Identification …ase functions)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	M.S.	Technician					Project-8283 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401				Non-Student Research Assistant					Project-8276 (Project 2.1 Inhibitors of … Therapeutics)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	PhD	Staff scientist (Doctoral level)					Project-8283 (Project 3.2 Novel Therapeu… Alphaviruses		NA

									)		
	N	Wooten, Julian	(b)(6)	(b)(6)	B.S.	Technician	(b)(4); (b)(6)		Project-8283 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	PHD	Co-Investigator			Project-8282 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	Botto, Sara			PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-8277 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	N	(b)(6); (b)(3):7 U.S.C. § 8401			PhD	Staff scientist (Doctoral level)			Project-8276 (Project 2.1 Inhibitors of … Therapeutics)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-8283 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	N	Hawman, David William			PHD,BS	Graduate Student (research assistant)			Project-8284 (Project 3.3 Novel Therapeu… Alphaviruses)		NA
	Y	DeFilippis, Victor Robert	(b)(6)		PHD,MS	Co-Investigator			Project-8277 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Quenelle, Debra			DVM,PhD	Co-Investigator			Project-8278 (Project 4.1 Identification …ase functions)		NA
	N	Madden, Emily	(b)(6)	(b)(6)	B.A.	Graduate Student (research assistant)			Project-8283 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	N	(b)(6); (b)(3):7 U.S.C. § 8401			PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-8285 (Project 4.2 Identification …ase functions)		NA

(b)(6)	Y	Hirsch, Alec	(b)(6)		BA,PHD	Co-Investigator	(b)(4); (b)(6)		Project-8275 (Project 1.1 Identification …ug Candidates)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401			PHD	Co-Investigator			Project-8275 (Project 1.1 Identification …ug Candidates)		NA
	Y					Co-Investigator			Project-8285 (Project 4.2 Identification …ase functions)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-8282 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N				PhD	Staff scientist (Doctoral level)			Project-8283 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	N	Carpentier, Kathryn Semmens		(b)(6)	BA,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-8284 (Project 3.3 Novel Therapeu… Alphaviruses)		NA
	N	VanBlargan, Laura A.			BA,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-8281 (Project 1.2 Identification …ug Candidates)		NA
	Y	Prichard, Mark Neal	(b)(6)		PHD,BS, MS	Co-Investigator			Project-8278 (Project 4.1 Identification …ase functions)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401			Ph.D.	Co-Investigator			Project-8282 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-8282 (Project 2.2 Inhibitors of … Therapeutics)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	N	(b)(6); (b)(3):7 U.S.C. § 8401			BS,PHD	Postdoctoral Scholar, Fellow, or Other			Project-8276 (Project 2.1 Inhibitors of …		NA

						Postdoctoral Position					Therapeutics)		
(b)(6)	N	Nguyen, T				Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	(b)(4); (b)(6)				Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	Y	Sheahan, Timothy P.	(b)(6)		PhD	Co-Investigator					Project-8282 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	Zhang, Rong			PHD,BS	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position					Project-8281 (Project 1.2 Identification …ug Candidates)		NA
	N	Ahmed, Kaleem S				Chemist					Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Ando, Takeshi	(b)(6)		MD/PhD	Adj. Research Associate Professor					Project-8277 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Bao, Donghui			PhD	Research Scientist					Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Bonin, Kiley	(b)(6)			Non OHSU Student Worker					Project-8277 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Bowers, Mary Wyatt	(b)(6)		MA	Admin Core Business Manager					Admin Core-8274 (Administrative Core - Core A)		NA
	N	Cabrera, Sara	(b)(6)		M.S.F.S.	Supervisor Compound Management					Core-8279 (Screening Core - Core B)		NA
	N	Chakraborty, Saibal				Associate Research Chemist					Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA

	N	Davis, Sara	(b)(6)	B.S.	Admin Core Administrative Coord.	(b)(4); (b)(6)		Admin Core-8274 (Administrative Core - Core A)		NA
	N	Deimler, R			Associate Research Chemist			Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Denton, Michael	(b)(6)	BS	Sr. Research Assistant			Project-8277 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401		MS	Project Manager			Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Ferreira-Martinez, Erika			Lab Aide			Project-8275 (Project 1.1 Identification …ug Candidates)		NA
	N	Hancock, Meaghan H	(b)(6)	BS	Research Asst. Professor			Project-8275 (Project 1.1 Identification …ug Candidates)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401		B.A.	Advanced Biologist			Core-8279 (Screening Core - Core B)		NA
	N	Keith, Kathy		MS	Research Lab Supervisor			Project-8278 (Project 4.1 Identification …ase functions)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			Senior Research Specialist			Project-8276 (Project 2.1 Inhibitors of … Therapeutics)		NA
	N	Maddadi, Nikhil			Associate Research Chemist			Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	B.S.	Advanced Bio IT Specialist			Core-8279 (Screening Core - Core B)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401		MS	Associate			Project-8285		NA

	(b)(6); (b)(3):7 U.S.C. § 8401				Biologist	(b)(4); (b)(6)		(Project 4.2 Identification …ase functions)		
	N	May, Nick		(b)(6)	B.S.	Professional Research Assistant		Project-8284 (Project 3.3 Novel Therapeu… Alphaviruses)		NA
	N	Parkins, Christopher	(b)(6)		M.S.	Research Associate		Project-8275 (Project 1.1 Identification …ug Candidates)		NA
	N	Poon, D			BS	Chemist		Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			MS	Associate Biologist		Project-8285 (Project 4.2 Identification …ase functions)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401				Advanced Bio IT Specialist		Core-8279 (Screening Core - Core B)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)		M.S.	Supervisor HTS Center		Core-8279 (Screening Core - Core B)		NA
	N				B.S.	Associate Biologist		Core-8279 (Screening Core - Core B)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			B.S.	Associate Biologist		Core-8279 (Screening Core - Core B)		NA
	N	Rodzinak, Kevin			BS	Research Chemist		Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Smith, Patricia	(b)(6)			Senior Research Associate		Project-8277 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Smith, V			PhD	Associate Research Chemist		Core-8280 (Medicinal Chemistry and Le…Core -		NA

											Core C)		
	N	Streblow, Aaron				Non OHSU Student Worker	(b)(4); (b)(6)				Project-8277 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)		M.S.	Research Scientist					Core-8279 (Screening Core - Core B)		NA
	N	Wei, H			PhD	Research Chemist					Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)			Research Specialist					Project-8282 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			M.S.	Biologist					Core-8279 (Screening Core - Core B)		NA
	N	Zhang, Wei			PhD	Research Scientist					Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
(b)(6)	Y	Augelli-Szafran, Corinne			PhD	Director of Organic Chemistry					Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)		MD	Project 2.1 Project Leader					Project-8276 (Project 2.1 Inhibitors of … Therapeutics)		NA
	Y				BA,PHD	Project 3.2 Consortium P.I.					Project-8283 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	Y	(b)(6); (b)(3):7 U.S.C. § 8401			PHD,BS	Screening Core B Project Leader					Core-8279 (Screening Core - Core B)		NA
	Y	(b)(6); (b)(3):7 U.S.C. § 8401			BA	Screening Core B Co-Project Leader					Core-8279 (Screening Core - Core B)		NA

(b)(6)	Y	Diamond, Michael S	(b)(6)	PHD,MD ,BA,MD, PHD	Project 1.2 Project Leader	(b)(4); (b)(6)		Project-8281 (Project 1.2 Identification …ug Candidates)		NA
	Y	Suto, Mark J		BS,PHD	PI			Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401		PHD,MS ,DVM	Project 4.2 Project Leader			Project-8285 (Project 4.2 Identification …ase functions)		NA
	Y	Nelson, Jay A		BS,PHD, BS,BOT H	Project 1.1 Leader			Project-8275 (Project 1.1 Identification …ug Candidates)		NA
	Y	Pathak, Ashish Kumar		PHD,MS ,BS	Co-Investigator			Core-8280 (Medicinal Chemistry and Le…Core - Core C)		NA
	Y	Baric, Ralph S		PHD,BS	Project 2.2 Project Leader			Project-8282 (Project 2.2 Inhibitors of … Therapeutics)		NA
	Y	Whitley, Richard J.		AB,MD	PD/PI			Project-8278 (Project 4.1 Identification …ase functions)		NA
	Y	Streblow, Daniel N		PHD,BS	Project 3.1 Project Leader			Project-8277 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	Y	Morrison, Thomas E		MA,PHD ,BA,BA	Project 3.3 Leader			Project-8284 (Project 3.3 Novel Therapeu… Alphaviruses)		NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

Yes

(b)(6); (b)(3):7 U.S.C. § 8401, co-PI for Core C, will (b)(6) Dr (b)(6); (b)(3):7 PI for Core C, will assume all of her responsibilities for the remainder of this project. (b)(6); (b)(3):7 U.S.C. § 8401 resigned September 1, 2016. Her position will be filled by a new hire.

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: Combined Other Support.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

Program Director/Principal Investigator:
(Last, first, middle)

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED

PHS 398 OTHER SUPPORT

WHITLEY, R.J.

ACTIVE

(b)(4)

HHSN272201100034C (Whitley, Kimberlin Co PI) 9/28/11-9/27/17
HHS-NIH-NIAID $3,385,690
An adaptive sequential study evaluating prevention of neonatal HSV: Detection of maternal shedding at delivery followed by preemptive antiviral therapy in exposed neonates.
In this project, a multi-institutional team of investigators, known as the Collaborative Antiviral Study Group (CASG), will validate the GeneXpert® HSV polymerase chain reaction (PCR) system by comparing it against standard quantitative PCR and routine viral culture.

(b)(4)

HHSN272201100035C (Whitley, Kimberlin Co PI) 9/28/11-9/27/17
HHS-NIH-NIAID $2,065,894
A Phase II 6 Weeks oral Valganciclovir vs Placebo in infants with Congenital CMV infection and hearing loss.
In this project, the CASG will identify infants and toddlers with SNHL and then will test these patients' DBS obtained during the neonatal period for CMV DNA by PCR.

(b)(4)

HHSN272201100036C (Whitley, Gnann Co PI) 9/28/11-9/27/17
HHS-NIH-NIAID $2,188,470
Natural History of Infection Caused by BK Virus (and other Opportunistic Viral Pathogens) in Renal and Renal-Pancreas Transplant Recipients
The primary objective of the study is to determine the natural history of kidney transplant patients with BKV viremia.

(b)(4)

HHSN272201100037C (Whitley, Kimberlin Co PI) 9/28/11-9/27/17
HHS-NIH-NIAID $1,779,753
A PK/PD and Resistance Evaluation of Intravenous Ganciclovir in Premature Infants
In this project, a multi-institutional group of investigators, known as the Collaborative Antiviral Study Group (CASG), will enroll premature subjects who are being treated clinically with intravenous ganciclovir for postnatally or congenitally acquired CMV disease.

(b)(4)

HHSN272201100038C (Whitley, Kimberlin Co PI) 9/28/11-9/27/19
HHS-NIH-NIAID $1,967,277
Adaptive study of CMX-001 in infants with Neonatal Herpes Simplex Virus (HSV)
In this project, a multi-institutional team of investigators, known as the Collaborative Antiviral Study Group (CASG), will define the pharmacokinetics (PK) and concentration response relationship of CMX001 in neonates with HSV CNS disease.

(b)(4)

1U19AI109680-03 (Whitley, PI) 3/1/14-2/28/19
HHS-NIH- NIAID $1,210,504 Admin core; $780,179-Proj 4
Center for Antiviral Drug Discovery and Development-UAB
Role: Administrative Core: PI, and Project Director; Project 4.0 – Influenza: Co-PI
Consortium which focuses on development of antiviral therapeutics for four major categories of emerging infectious diseases including Flaviviruses, Corona viruses, Alphaviruses and Influenza

(b)(4)

U54TR001368-01 (Kimberly) 9/01/15 – 8/31/20
NIH/NCATS $6,324,075 (UL1, KL2, TL1)
UAB Center for Clinical and Translational Science (CCTS)
The UAB CCTS will enhance human health by driving scientific discovery and dialogue across the bench, bedside and community continuum. The CCTS supports this overall mission in a highly integrative network of relationships. Five strategic priorities are: 1) enhancing research infrastructure; 2) promoting investigator education, training and development; 3) accelerating discovery across the T1 interface; 4) expanding value-added partnerships; and 5) building sustainability.
Role: Co-Investigator, Project Leader

(b)(4)

UM1 AR065705 (Curtis, Winthrop, MPI) 9/01/14 – 8/31/19
NIH/NIAMS $437,212 Annual Direct Costs
Safety and Effectiveness of Live Zoster Vaccine in Anti- TNF Users (VERVE)
The Varicella zostER VaccinE (VERVE) trial is a randomized, double-blind, placebo-controlled large pragmatic trial to evaluate the immunogenicity, safety, and longer-term effectiveness of the live HZ vaccine in arthritis patients receiving anti-TNF therapy
Role: Advisor

(b)(4)

HHS/NIH/NIAID (Kimberlin, PI) 5/01/16 – 04/28/21
$9,975,877
A Phase II, Single-Stage, Single Arm Investigation of Oral Valganciclovir Therapy in Infants with Asymptomatic Congenital Cytomegalovirus Infection.
Role: Co-Investigator

COMPLETED

(b)(4)

N01-AI-30025 (Whitley, PI) 8/1/03-12/01/13
NIH-NIAID $3,719,919
Clinical Trials for Antiviral Therapies
This contract serves to facilitate the development of promising therapies for treating severe, acute, and chronic human viral diseases that are deemed medically and scientifically important by the NIH-NIAID. This program will facilitate advances in clinical antiviral therapy by rigorously evaluating the efficacy and safety of new therapeutic regimens for serious viral diseases in adult and pediatric patient populations.

(b)(4)

3 U54 A1057157 (Sparling-UNC PI) 3/01/09–2/28/14
NIH $31,000
SERCEB Southeast Regional Centers for Excellence for Biodefense
This grant is jointly submitted by investigators in the Southeastern United States and researches new ways to contribute to the national effort in biodefense, as well as to study emerging infectious diseases that threaten both our country and our world.
Role: Co-Investigator.

(b)(4)

2K12HD043397-09 (Stagno, PI) 3/10/08-11/30/13
NIH $349,821
Pediatric Physician Scientist in Translational Molecular Biology
The goal is to enhance the mentored research experience with a foundation of research techniques and approaches. Role: Co-Investigator.

(b)(4)

1U54 RR024376-05 (Kimberly, PI) 7/1/08-4/30/14

NIH $232,220
UAB Center for Clinical and Translational Science (CCTS)
The UAB Center for Clinical and Translational Science (CCTS) will transform the UAB environment by building productive and efficient interdisciplinary research teams through educational ingenuity, regulatory reorganization, resource coordination, and methodological innovation. Its mission is to develop a transformative infrastructure that spans the spectrum from pre-clinical research to bench-to-bedside translation (T1 research) to community implementation (T2 research).
Role: Co-Investigator, Project Leader

1UL1RR025777-01(Kimberly, PI) 05/01/14-4/30/15 (b)(4)
NIH $80,109
UAB Center for Clinical and Translational Science (CCTS) supplement
The UAB Center for Clinical and Translational Science (CCTS) Drug Discovery project continuation which seeks to provide program for academic drug development in fulfillment of the overall mission to develop a transformative infrastructure that spans the spectrum from pre-clinical research to bench-to-bedside translation (T1 research) to community implementation (T2 research).
Role: Co-Investigator, Project Leader

5P01-CA 071933-15 (Whitley, PI) 7/1/09-6/30/15 (b)(4)
NIH-NCI $218,869
Engineered HSV for the Treatment of Malignant Glioma
The long-term objective of this program project grant, in collaboration with Dr. Bernard Roizman at the University of Chicago, was the design and testing of novel recombinant herpes simplex viruses as vectors of noxious genes for the selective destruction of human glioma cells.

For New and Competing Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

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Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL: CORINNE E. AUGELLI-SZAFRAN
ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project *(or Subproject)* The major goals of this project are…	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)

OVERLAP *(summarized for each individual)*

ACTIVE –

29XS124TO21, Xu (PI) 07/01/2016 –10/31/2016 (b)(4)
NIH, Leidos Biomedical Research $ 255,046

Inhibitors of the Artemis Endonuclease for Cancer Chemotherapy. Structural biology, purification and crystallization efforts on truncates of the Artemis protein as well as co-crystallization of identified small molecules of inhibitors with protein truncates will be investigated to assist with identification of a novel therapy for cancer chemotherapy.

1U19AI109680, Whitley (PI) 03/01/2015 – 02/28/2019 (b)(4)
NIAID $1,046,665
UAB 000502793-011
Antiviral Drug Discovery and Development Center. The goals of this NIAID program are:
Development of antiviral drugs for the treatment of emerging and reemerging infections. Specifically, the focus will be on flaviviruses, alphaviruses, corona viruses and influenza. The goal is to identify compounds working through mechanisms that affect viral replication and develop these leads in a translational manner to new human therapeutics. As described above, each of the projects is focused on a viral family deemed critical to NIAID's focus on Emerging and Re-emerging Infectious Diseases related to biodefense. Role: Co-Investigator

HHSN272201400010I, Ptak (PM) 08/01/2015 – 7/31/2017 (b)(4)
NIAID Task Order16 $105,482

In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature.
Role: Co-Investigator.

HHSN272201400010I, Ptak (PM) 09/01/2014 – 7/31/2017 (b)(4)
NIAID Task Order 8 $284,267

In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature.
Role: Co-Investigator

(b)(4) Robinson (PI) 6/01/2015 – 06/30/2016 (b)(4)
$26,034

Goal: Development of Inhibitors of the Tau-Fyn Interaction for the Treatment of Alzheimer's disease. This project aims to develop inhibitors of the Tau–Fyn interaction for treatment of Alzheimer's disease and represents a new therapeutic strategy for the disease.
Role: Co- Investigator

HHSN272201400010I, Ptak (PM) 08/01/2016 – 7/31/2018 (b)(4)
NIAID Task Order 20 $108,581

In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature.
Role: Co-Investigator

HHSN272201400010I, Ptak (PM) 08/01/2016 – 7/31/2018 (b)(4)
NIAID Task Order 22 $105,709

In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature.
Role: Co-Investigator

HHSN272201400010I, Ptak (PM) 09/15/2016 – 9/14/2018 (b)(4)
NIAID Task Order 24 $ 326,951

In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature.
Role: Co-Investigator

PENDING

OVERLAP - None

OTHER SUPPORT

BARIC, RALPH S.

ACTIVE:

U19 AI 107810 (PI: Baric) 06/21/13-05/31/18 (b)(4)
NIH/NIAID $1,572,931
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

U19-AI100625 (PI: Baric/Heise-MPI) 08/05/12-07/31/17 (b)(4)
NIH/NIAID $3,580,599
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

00008956 (PI: De Silva) 07/29/15-06/30/17 (b)(4)
UCB/NIH $279,165
Protective immunity following dengue virus natural infections and vaccination
We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).
Role: Co-Investigator

R01 AI 107731 (PI: De Silva) 08/05/13-07/31/17 (b)(4)
NIH/NIAID $621,124
Molecular Basis of Dengue Virus Neutralization by Human Antibodies
These studies proposed here are directly relevant to developing simple assays to predict the performance of the leading dengue vaccine candidates and also for developing the next generation of safe and effective dengue vaccines.
Role: Co-Investigator

R01 AI108197 (MPI: Denison/Baric) 08/01/13-07/31/17 (b)(4)
Vanderbilt University/NIH/NIAID $187,635
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination frequencies.

U19-AI106772-01 (PI: Kawaoka) 06/01/13-05/31/17 (b)(4)
Univ of Wisconsin/NIH $55,729
MERS-CoV Supplement for OMICs Proposal
The proposed studies will provide a more detailed look at the intracellular environment by taking “snapshots” of the lipids, metabolytes, and proteins present during viral infection time courses. These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window.
Role: Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4)
UAB/NIH/NIAID $304,371
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Co-Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4)
Columbia/NIH/NIAID $584,891
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Project Leader, Consortium PI

246823 (PI: Baric) 01/27/15-12/30/16 (b)(4)
PNNL/DHS $205,569
The Generation of Predictive Models of Viral Pathogenesis
Using advances in transcriptomics, proteomics, and metabolomics, we will identify changes in the virus-host interaction expression networks associated with DENV infection of Aedes aegypti cells or human immune cells in vitro, the latter model after natural receptor-mediated or after ADE mediated entry processes.

Not assigned (PI: deSilva) 11/05/14-09/30/17 (b)(4)
Johns Hopkins U/Gates Foundation $726,915
The dengue human infection model: Defining correlates of protection and advancing vaccine development
The goal of these studies conducted by the Baric laboratory are to use recombinant dengue viruses encoding multiple homotypic neutralizing sites from multiple strains, as well as a collection of null mutants, to characterize the homotypic immune response elicited in humans following natural infection and after challenge in GSK DENV tetravalent vaccinated individuals. This grant has been funded by Gates.
Role: Co-Investigator

R01 AI110700 (PI: Baric) 04/20/15-03/31/20 (b)(4)
NIH/NIAID $613,691
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

(b)(4) (PI: Baric) 01/08/16-01/07/19 (b)(4)
$1,243,048
In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines
To provide expertise in molecular virology required for creating recombinant dengue viruses for in vitro and in vivo testing.

(b)(4) (PI: deSilva) 02/29/16-02/28/17 (b)(4)
$212,800
UNC (b)(4) Pilot study to characterize human antibody response to tetravalent dengue vaccine- phase 3.
To perform experiments to produce recombinant dengue viruses using DENV1, 2, 3 and 4 infectious clones, conducting neutralization assays, culturing viruses and performing other phenotypic analyses.
Role: Co-Investigator

R01-AI125198 (PI: deSilva) 05/04/16-04/30/21 (b)(4)
NIH/NIAID $1,153,997

Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy
Dengue is the most significant mosquito transmitted viral infection of humans. Vaccination is a feasible solution to prevent and control dengue. Although dengue vaccines are under development, we do not know the specific properties of antibodies induced by vaccines that are likely to protect from infection. In this project investigators from the University of North Carolina and (b)(4) a leading dengue vaccine developer, will collaborate to define properties of antibodies induced by the Sanofi vaccine that correlate with protection. The main goal of the project is to develop new assays to support the current global effort to develop dengue virus vaccines. Role: Co-Investigator

(b)(4)

60045042 (PI: Baric) 02/01/15-01/31/18
Ohio State Univ/USDA $44,804

Molecular attenuation mechanisms of porcine epidemic diarrhea virus in pigs
Reverse genetic strategies are used to construct a panel of live attenuated porcine epidemic diarrhea recombinant viruses for in vivo pathogenesis studies and in vitro biological characterization. We test rationale vaccine strategies to protect new born piglets against this devastating porcine epidemic virus.

(b)(4) (PI: Baric) 06/23/16-06/22/18
$1,066,500

(b)(4)

Breadth of Blockade Antibody Responses Following Norovirus Vaccination
To conduct a project as an agreement in which Dr. Ralph Baric will test Takeda provided serum samples for cross-strain blockade antibody responses.

(b)(4)

0258-3962 (PI: Lim) 09/30/11-02/28/17
Mount Sinai/NIH $166,793

MERS-CoV Mouse Model for Vaccine and Therapeutic Testing
To: 1) confirm that the non-conserved region of mouse DPP4 is responsible for its inability to serve as a MERS-CoV entry receptor, 2) use a powerful new in vivo site directed mutagenesis approach to humanize the murine DPP4, 3) test whether mice carrying the humanized DPP4 receptor will support MERS-CoV replication and disease within the lungs. Role: Subcontract PI

(b)(4)

N005402801 (PI: Li) 06/07/16-05/2831/17
Univ Minn/NIH $120,384

Receptor recognition and cell entry of coronaviruses
To investigate how CoVs explore host receptors and host proteases for regulation of their host range, cross-species transmission, tissue tropism, and pathogenesis. Role: Subcontract PI

(b)(4)

684K644 (PI: Sims) 06/01/16-05/31/17
Univ of Wisconsin/NIH $200,000

Systems Virology of MERS-CoV in vivo
The major goal of this award is to define host cell gene networks and pathways that are modulated following infection in our newly developed lethal mouse model of MERS-CoV. Role: Investigator

PENDING: None

OVERLAP: None

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel

PHS 398/2590 OTHER SUPPORT

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Format

NAME OF INDIVIDUAL
ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project *(or Subproject)* The major goals of this project are…	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)

OVERLAP *(summarized for each individual)*

Samples

(b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401

PHS 398/2590 OTHER SUPPORT

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Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

(b)(6); (b)(3):7 U.S.C. § 8401

OTHER SUPPORT FOR ALL KEY PERSONNEL – OREGON HEALTH & SCIENCE UNIVERSITY

DEFILIPPIS, V

ACTIVE

HHSN272201400055C (Nelson) 9/30/2014 – 9/29/2019 (b)(4)
Adjuvant Discovery Program $1,787,755
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity
The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses.
Role: Co-Investigator / Project Lead on awarded supplement

(b)(4) (Picker) 7/28/2014 – 8/31/2019 (b)(4)
$6,144,126
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine
The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (composed of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Leader

5 U19 AI109680-02 (Whitley) 3/01/2014 – 2/28/2019 (b)(4)
NIH/NIAID $299,955
Antiviral Drug Discovery and Development Center
Project 3B: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
The main goal of this project is to develop novel nucleoside and nucleotide inhibitors directed against Alphaviruses including Chikungunya virus and Venezuelan Equine Encephalitis virus.
Role: Project 3B Co-Investigator

Departmental Support (b)(4)
Vaccine and Gene Therapy Institute

INACTIVE

5 U54 AI081680-05 (Nelson) 3/01/2011 – 2/28/2015 0.0 calendar
NIH/NIAID (no cost extension)
Pacific NorthWest Regional Center of Excellence Developmental Research Project
Role of cytokines in Chikungunya virus-associated disease
The goal of this project is an understanding of the contribution of these host-virus interactions to CHIKV-associated disease. CHIKV is a re-emerging arthritogenic mosquito-borne RNA Alphavirus. Infection results in serum induction of multiple proinflammatory cytokines including type I interferons (IFN).
Role: Developmental Research Project (DP005) PI

OVERLAP No overlap

OTHER SUPPORT

(b)(6); (b)(3):7 U.S.C. § 8401

PHS 398/2590 OTHER SUPPORT

(b)(6); (b)(3):7 U.S.C. § 8401

OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

HIRSCH, A

ACTIVE

5 U19 AI109680-03 (Whitley) 3/01/2014 – 2/28/2019 (b)(6)
NIH/NIAID $337,039
Antiviral Drug Discovery and Development Center: Project 1: Identification and Development of Anti-Flavivirus Lead Drug Candidates
The overall goal of this proposal is to discover and characterize compounds with broad anti-flavivirus activity.
Role: Co-Investigator

HHSN27201400055C (Nelson) 9/30/2014 – 9/29/2019 (b)(6)
NIH/NIAID $1,787,755
Adjuvant Discovery Program
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity
The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses.
Role: Co-Investigator

(b)(4)
Departmental Support
Vaccine and Gene Therapy Institute

1 R21 HD091032-01 (Streblow) 9/01/2016 – 8/31/2018 (b)(4)
NIH $150,000
Development of a NHP Model for Determining the Causal Relationship Between Zika Virus Infection During Pregnancy and Fetal Microcephaly
The goal of this project is to develop a NHP model of Zika virus infection and fetal disease.
Role: Co-Investigator

INACTIVE

BAA-NIAID-DAIT-HIHAI2010085 (Nikolich-Zugich PI) 5/16/2011 – 5/15/2016 (b)(4)
Subcontract Y562709 on NIH Contract No. HHSN272201100017C
NIH/NIAID $195,051
Protective Immunity in Special Populations: Interface between Innate and Adaptive Immunity
The major goal of this subcontract is to determine the role of miRNAs on immune function in the context of aging.
Role: Co-Investigator

5 R21 AI101282-02 (Hirsch) 6/01/2012 – 5/31/2015 (b)(4)
NIH/NIAID $120,846 (no-cost extension)
Evaluation of Host miRNAs as Therapeutics against Encephalitogenic Flaviviruses
The overall goal of this proposal is to use cellular microRNAs (miRNAs) as potential targets of therapeutic intervention for the neurotropic flaviviruses West Nile virus (WNV) and Japanese encephalitis virus (JEV).
Role: PI

5 U54 AI 081680-05 (Nelson) 4/20/2009 – 2/28/2015 0.00 calendar
NIH/NIAID $5,688,792 (no-cost extension)
Pacific Northwest Regional Center of Excellence – Developmental Project: The role of microRNAs in flavivirus replication (DP 006)
The overall goal of this proposal is to elucidate how the observed changes in miRNA expression affect WNV replication and pathogenesis as well as to extend this analysis to the dengue viruses, which are also members of the flavivirus family.
Role: PI of Developmental Project

(b)(4)

(b)(4)

(Hirsch) 9/15/2012 – 7/10/2014
$113,200

(b)(4)

Development of a Thienopyradine Compound as an Anti-Dengue Virus Therapeutic

The overall goal of this proposal is to identify the compound with DENV-inhibitory activity in high-content replication assay.

Role: PI

OVERLAP – No overlap

OTHER SUPPORT

MORRISON, T.

ACTIVE

U19 AI109680 (Whitley) 3/1/2014 – 2/28/2019 (b)(4)
NIH/NIAID $149,913
Title: Antiviral Drug Discovery and Development Center
The major goals of this project are to i) identify small molecules capable of inhibiting replication of diverse members of the *Alphavirus* genus and ii) test candidate molecules for prophylactic and therapeutic efficacy against chronic chikungunya (CHIKV) infection and joint disease.

R01 AI108725 (Morrison) 7/1/2014 – 6/30/2019 (b)(4)
NIH/NIAID $250,000
Title: Mechanisms of Immune Suppression During Arthritogenic Alphavirus Infections
The major goals of this project are to i) define mechanisms by which iNOS and Arg1-mediated production of peroxynitrite suppresses T cell responses to infection and ii) to define cytokine signaling pathways that promote the immunosuppressive activity of macrophages and impair control of Ross River virus infection in musculoskeletal tissues.

R01 AI123348 (Dermody, Morrison, Diamond) 4/1/2016 – 3/31/21 (b)(4)
NIH/NIAID $114,301
Title: Chikungunya Virus Replication and Pathogenesis
The major goals of this project are to i) determine how CHIKV E2 engages specific GAGs and the role of GAG binding in CHIKV pathogenesis, ii) discover mechanisms by which COPI transport promotes CHIKV infection, and iii) identify the cell types infected by CHIKV in mice that contribute to acute and chronic disease.

PENDING

(b)(4)

OVERLAP
None

OTHER SUPPORT FOR ALL KEY PERSONNEL – OHSU

NELSON, JA

ACTIVE

5 P01 AI094417-05S1 (Picker) 7/15/2011 – 6/30/2017 (b)(4)
NIH/NIAID $219,865 (1-year extension)
Development of an Effector-Memory T Cell AIDS Vaccine (Project 2: Attenuation of CMV Vector Pathogenicity and Transmission by Altering Viral Tropism)

The goal of this project is to determine whether genetically modifying CMV to limit its ability to replicate in cell types associated with disease and transmission, while retaining its ability to persist in cells important for eliciting immunity, will lead to a safe and effective vector for an HIV/AIDS vaccine. In this Program, we will modify CMV vectors and/or use complementary heterologous vaccines with CMV vectors to both increase the potency of CMV/SIV vectors so as to achieve rates of protection closer to 100% of vaccines, and reduce the pathogenicity and shedding potential of CMV vectors (while retaining immunogenicity), so as to achieve an effective vaccine that is safe enough for use in a general human population.
Role: Project 2 PI

4 R01 AI021640-31 (Nelson) 12/01/1984 – 1/31/2018 (b)(4)
NIH/NIAID $299,774
Molecular Aspects of Cytomegalovirus Latency

The long-term goal of this project is to develop an understanding of the cellular and molecular mechanisms of human cytomegalovirus (HCMV) persistence in the host. This project will use HCMV miRNA mutants as well as miRNA inhibitory molecules in an in vitro CD34+ human progenitor cell system and a humanized mouse model to examine the role of the viral miRNAs in latency and reactivation.
Role: PI

HHSN272201400055C (Nelson) 9/30/2014 – 9/29/2019 (b)(4)
NIH/NIAID $1,787,755
Adjuvant Discovery Program
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity

The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses.
Role: PI

1 R01 AI120619-01A1 (Britt/Nelson) 4/01/2016 – 3/31/2021 (b)(4)
NIH/NIAID $229,129
HCMV miRNA Regulation of Secretion and Formation of the Viral Assembly Compartment

The major goals of this project are to elucidate the mechanisms through which human cytomegalovirus (HCMV) microRNAs (miRNAs) restructure the endocytic/secretory pathway to regulate secretion of cytokines, recycle proteins from the cell surface and form the viral assembly compartment (VAC).
Role: Co-PI (Multiple PI submission)

1 R13 AI126606-01 (Nelson) 7/01/2016 – 6/30/2017 (b)(4)
NIH/NIAID $8,000
International Herpesvirus Workshop

The project supports travel and registration fees for postdoctoral fellows and graduate students to attend the annual International Herpesvirus Workshops (IHW).
Role: PI

(b)(4) (Picker) 7/28/2014 – 8/31/2019 (b)(4)
$6,144,218
Collaboration for AIDS Vaccine Discovery (CAVD)
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine

The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (comprised of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Leader; Outcomes 2.2 and 2.3

(b)(4)

5 U19 AI109680-03 (Whitley) 3/01/2014 – 2/28/2019
NIH/NIAID $274,539
Antiviral Drug Discovery and Development Center: Project 1: Identification and Development of Anti-Flavivirus Lead Drug Candidates
This project is designed to identify and develop small molecule anti-viral therapeutics against two medically important flaviviruses--dengue virus and West Nile virus. Furthermore, we will emphasize the development of drugs that show activity against multiple flaviviruses, and possibly other virus families as well.
Role: Site PI; Project 1

(b)(4)

5 R01 CA179921-02 (Moses) 5/01/2015 – 4/30/2020
NIH/NCI $228,750
Heme Oxygenase-1 as a Tumor Factor and Therapeutic Target for Kaposi Sarcoma
The major goals of this project are to characterize the role of the host enzyme heme oxygenase-1 (HO-1) in KSHV pathogenesis and Kaposi sarcoma (KS), and to determine if HO-1 is a valid therapeutic target for KS.
Role: Co-Investigator

(b)(4)

8 P51 OD011092-57 (Robertson) 5/01/2014 - 4/30/2019
NIH/OD (salary support only)
Support for Oregon National Primate Research Center
Role: Senior Scientist, Division of Pathobiology and Immunology

(b)(4)

Departmental Support
Vaccine and Gene Therapy Institute

OVERLAP

No active or pending application has scientific or budgetary overlap. If a pending grant is awarded, effort will be adjusted between the OHSU support, existing grants and the new grant, following NIH guidelines.

INACTIVE

(b)(4)

HHSN272201100017C (Nikolich-Zugich) 5/16/2011 – 5/15/2016
NIH/NIAID
Protective Immunity in Special Populations: Interface Between Innate and Adaptive Immunity
This contract is a renewal of ongoing studies of our group with Dr Nikolich-Zugich to characterize defects in the aged immune system.
Role: Subcontract PI

(b)(4)

1 R56 AI105062-01 (Goodrum) 9/01/2013 – 7/31/2015
NIH/NIAID (no-cost extension)
Antagonistic Viral Determinants Regulating the Outcome of Infection
The goal of this work is to determine the role of virus-host interactions identified by Dr. Goodrum in viral persistence. Dr. Goodrum will provide recombinant viruses. We will perform the experiments in NSG mice we create and harvest tissues for analysis.
Role: Subcontract PI

(b)(4)

(b)(4) (Picker) 9/15/2011 – 6/30/2015
(no-cost extension)
Collaboration for AIDS Vaccine Discovery (CAVD)
Development of an Attenuated CMV Vector for an HIV/AIDS Vaccine
The goal of this project is to construct HCMV/HIV vector homologues of the interim and optimized designs in Objective 2 and to determine whether these homologues exhibit analogous replication, tropism characteristics and level of insert expression compared to the in vivo-validated RhCMV/SIV version. Assessment of the

interim vector designs will provide go/no-go criteria for further development, as only designs that work in the context of HCMV will move forward. Assessment of the final optimized version will provide crucial information for regulatory approval and clinical translation. The ultimate aim is to develop a safe and effective HIV/AIDS vaccine for use in high burden countries.
Role: Project Leader, Objective 3

1 R13 AI113945-01 (Nelson) 7/01/2014 – 6/30/2015 (b)(4)
NIH/NIAID $8,000
International Herpesvirus Workshop
The project supports travel and registration fees for postdoctoral fellows and graduate students to attend the annual International Herpesvirus Workshops (IHW).
Role: PI

5 U54 AI081680-05 (Nelson) 4/20/2009 – 2/28/2015 (b)(4)
NIH/NIAID (no-cost extension)
Pacific Northwest Regional Center of Excellence in Biodefense and Emerging Infectious Diseases
The goal of the PNWRCE is to identify age-related immune system defects to develop new vaccines and supplemental therapies to enhance protection of individuals to NIAID Category A-C pathogens. A second goal of this center is to use systems genetic, chemical, and proteomics approaches to identify therapeutic targets for biodefense and emerging diseases.
Role: PI

1 R13 AI108033-01 (Nelson) 7/15/2013 – 6/30/2014 (b)(4)
NIH/NIAID $4,000
International Herpesvirus Workshop
The project supports travel and registration fees for postdoctoral fellows and graduate students to attend the 38th International Herpesvirus Workshop (IHW) in Grand Rapids, Michigan, United States, in 2013.
Role: PI

Other Research Support **Mark Prichard, UAB**

ACTIVE

HHSN272201100016I (Prichard) 6/01/2011 – 05/31/2018
NIH/NIAID (Base Contract)

The goals of this contract are to evaluate compounds against human DNA viruses

HHSN27200013 (Prichard) 9/16/16– 9/15/17 (b)(4) months (b)(4)
NIH/NIAID TOR B24: In Vitro Assessment for Anti $1,064,375
The goal of this contract is to evaluate compounds against human DNA viruses

HHSN2722010000271 (Quenelle) 9/30/15– 9/29/16 (b)(4) months (b)(4)
NIH/NIAID $614,144
Animal models for herpes simplex virus and human cytomegalovirus

HHSN272201100034C (Whitley 1) 9/28/11-9/27/16 (b)(4) months (b)(4)
NIH/NIAID $3,385,690
Adaptive sequential study evaluating prevention of neonatal HSV: Detection of maternal shedding.

HHSN272201100035C (Whitley 2) 9/28/11-9/27/16 (b)(4) months (b)(4)
NIH/NIAID $2,065,894
A Phase II 6 Weeks Oral Valganciclovir versus Placebo in Infants with Congenital CMV Infection.

HHSN272201100036C (Whitley 3) 9/28/11-9/27/15 (b)(4) months (b)(4)
NIH/NIAID $2,188,470
Safety Tolerability and Pharmacokinetics of CMX001 in Renal Transplant Recipients with BKV.

HHSN272201100037C (Whitley 4) 9/28/11-9/27/16 (b)(4) months (b)(4)
NIH/NIAID $1,779,753
A Pharmacokinetic/Pharmacodynamic and Resistance Evaluation of Intravenous Ganciclovir in Infants.

HHSN272201100038C (Whitley 5) 9/28/11-9/27/16 (b)(4) months (b)(4)
NIH/NIAID $1,967,277
Adaptive study of CMX001 in infants with neonatal herpes simplex virus (HSV)

2R44AI100401-03 SBIR Phase 2 (TSRL subcontra 12/1/14-11/30/17 (b)(4) months (b)(4)
NIH/NIAID $147,933
The goal of the research is to evaluate broad spectrum antiviral drugs against the DNA viruses

1U19AI 109680-01 (Whitley 6) 1/1/14-12/31/18 (b)(4) months (b)(4)
NIH/NIAID $34,138,453
The goal of the research is to support influenza studies for CETR Grant

(b)(4) 10/14/14-10/13/17 (b)(4) months (b)(4)
The goal of the research is In Vitro Antiviral Testin $91,610

(b)(4) 8/22/16-12/21/17 (b)(4) months (b)(4)

Evaluation of (b)(4) Compounds for Antiviral Activ $100,000

OVERLAP None

HHSN27200013 (Prichard) (b)(4) months (b)(4)

HHSN2722010000271 (Quenelle) (b)(4) months (b)(4)

HHSN272201100034C (Whitley 1) (b)(4) months (b)(4)

HHSN272201100035C (Whitley 2) (b)(4) months (b)(4)

HHSN272201100036C (Whitley 3) (b)(4) months (b)(4)

HHSN272201100037C (Whitley 4) (b)(4) months (b)(4)

HHSN272201100038C (Whitley 5) (b)(4) months (b)(4)

v

OTHER SUPPORT

SHEAHAN, TIMOTHY

ACTIVE:

U19AI107810 (PI: Baric) 06/21/13-05/31/18 (b)(4)
NIH/NIAID $2,027,645
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses, which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.
Role: Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4)
UAB/NIH/NIAID $1,611,425
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4)
Columbia/NIH/NIAID $2,999,060
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Investigator

R01 AI110700 (PI: Baric) 04/01/15-03/31/20 (b)(4)
NIH $3,683,050
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.
Role: Investigator

R01 AI108197 (MPI: Denison/Baric) 08/01/13-07/31/17 (b)(4)
Vanderbilt University/NIH/NIAID $450,129
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination frequencies.
Role: Investigator

OVERLAP:

If other awards are made, Dr. Sheahan will reduce his percent effort accordingly.

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

SMITH, JL

ACTIVE

5 U19 AI109680-03 (Whitley) 3/01/2014 – 2/28/2019 (b)(4)
NIH/NIAID $274,539
Antiviral Drug Discovery and Development Center: Project 1: Identification and Development of Anti-Flavivirus Lead Drug Candidates

The overall goal of this proposal is to discover and characterize compounds with broad anti-flavivirus activity.

Role: Co-Investigator

HHSN27201400055C (Nelson) 9/30/2014 – 9/29/2019 (b)(4)
NIH/NIAID $1,787,755
Adjuvant Discovery Program
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity

The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses.

Role: Staff Scientist

OVERLAP – No overlap

INACTIVE

None

OTHER SUPPORT FOR ALL KEY PERSONNEL – OREGON HEALTH & SCIENCE UNIVERSITY

STREBLOW, D

ACTIVE

1 R01 AI116633-01A1 (Streblow) 3/01/2016 – 2/28/2021 (b)(4)
NIH/NIAID $125,000
Characterizing the Role of CMV Latency in Solid Organ Transplant Rejection
The main goal of this project is to determine the mechanisms of cytomegalovirus-mediated solid organ transplant rejection.

5 U19 AI109680-03 (Whitley) 3/01/2014 – 2/28/2019 (b)(4)
NIH/NIAID $274,539
Antiviral Drug Discovery and Development Center
Project 3B: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
The main goal of this project is to develop novel nucleoside and nucleotide inhibitors directed against Alphaviruses including Chikungunya virus and Venezuelan Equine Encephalitis virus.
Role: Project Leader; Project 3B

(b)(4) (Picker) 7/28/2014 – 8/31/2019 (b)(4)
$6,144,126
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine
The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (composed of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Manager; Outcome 5 and Outcome 8

(b)(4) (Picker) 9/22/2014 – 9/30/2017 (b)(4)
$1,578,446
MHC II- and MHC E-restricted CD8+ T Cells and Control of HIV
The goal of this project is to provide fundamental research on a new type of vaccine-elicited CD8+ T cell immunity with the potential to control and clear HIV, and therefore to enable development of a safe and effective HIV/AIDS vaccine for use in high burden countries.
Role: Project Manager; Outcome 8 Activity 4.3

5 P50 CA097186-12 6/01/2015 – 12/31/2016 (NCE) (b)(4)
Fred Hutchinson Cancer Research Center/Prostate Cancer Research Institute/
Knight Cancer Institute-NIH/NCI $50,000
PNW Prostate Cancer SPORE Developmental Research Program (DRP), Pilot Project Award
CMV-vectored Prostate Cancer Vaccine
We have generated a novel vaccine platform using cytomegalovirus as a vector that induces astonishing levels and breadth of effector memory T cell responses that we will use to attempt to break tolerance to self antigens associated with prostate cancer, in order to generate a curative prostate cancer immunotherapy.
Role: PI

Departmental Support (b)(4)
Vaccine and Gene Therapy Institute

1 R21 HD091032-01 (Streblow) 9/01/2016 – 8/31/2018 (b)(4)
NIH $150,000
Development of a NHP Model for Determining the Causal Relationship Between Zika Virus Infection During Pregnancy and Fetal Microcephaly
The goal of this project is to develop a NHP model of Zika virus infection and fetal disease.

INACTIVE

1 R41 AI109927-01A1 (Streblow/Bruening) 4/01/2014 – 3/31/2016 (b)(4)
TomegaVax, Inc.-NIH/NIAID Advanced Technology STTR (No-cost extension)
CMV Vectored Herpes Simplex Vaccine
TomegaVax (PI Bruening) will generate wild type and spread-deficient murine CMV vaccine vectors that express fragments of HSV-2 ICP0 and ICP4. The Streblow Lab will test the immunogenicity and protective efficacy of CMV vaccine vectors directed against HSV-2 in mice.
Role: Co-PI

R56 AI116633-01 (Streblow) 9/02/2015 – 8/31/2016 (b)(4)
NIH/NIAID $250,000
Characterizing the Role of CMV Latency in Solid Organ Transplant Rejection
The main goal of this project is to determine the mechanisms of cytomegalovirus-mediated solid organ transplant rejection.
Role: PI

(b)(4) (Picker) 9/15/2011 – 6/30/2015 0.0 calendar
(no cost extension)
Collaboration for AIDS Vaccine Discovery (CAVD)
Development of an Attenuated CMV Vector for an HIV/AIDS Vaccine
The goal of this project is to construct HCMV/HIV vector homologues of the interim and optimized designs in Objective 2 and to determine whether these homologues exhibit analogous replication, tropism characteristics and level of insert expression compared to the *in vivo*-validated RhCMV/SIV version. Assessment of the interim vector designs will provide go/no-go criteria for further development, as only designs that work in the context of HCMV will move forward. Assessment of the final optimized version will provide crucial information for regulatory approval and clinical translation. The ultimate aim is to develop a safe and effective HIV/AIDS vaccine for use in high burden countries.
Role: Assistant Scientist

Defined Research Agreement (Früh/Streblow) 7/01/2014 – 6/30/2015 (b)(4)
OHSU Knight Cancer Institute $50,000
CMV-Vectored Cancer Vaccines
The goal of this project is to use CMV vectors to break tolerance to the prostate cancer associated protein PAP and by doing so elicit protective immune responses to the development of prostate cancer and metastatic disease, which would save tens of thousands of lives per year in the U.S. alone and would limit the need for invasive surgery or chemotherapies that have undesired side-effects and consequences.
Role: Co-PI

R01 AI089591 (Diamond/Streblow) 9/01/2014 – 5/31/2015 (b)(4)
NIH/NIAID (Administrative Supplement) $114,255
Antibody-Based Therapy of Chikungunya Virus
The major goal of this project is to generate an antibody therapy to control Chikungunya virus disease.
Role: Subcontract PI

1 R41 AI109927-01A1 (Streblow/Bruening) 4/01/2014 – 3/31/2015 (b)(4)
NIH/NIAID $95,212
CMV Vectored Herpes Simplex Vaccine
TomegaVax (PI Bruening) will generate wild type and spread-deficient murine CMV vaccine vectors that express fragments of HSV-2 ICP0 and ICP4. The Streblow Lab will test the immunogenicity and protective efficacy of CMV vaccine vectors directed against HSV-2 in mice.
Role: Co-PI

5 U54 AI081680-05 (Nelson) 4/20/2009 – 2/28/2015 0.0 calendar
NIH/NIAID (no cost extension)
Pacific Northwest Regional Center of Excellence Developmental Research Project:
Identification of Age-Related Defects to CHIKV Infections in a NHP Model

This project seeks to uncover the immunological and virological basis underlying increased Chikungunya virus (CHIKV) disease severity in the elderly. CHIKV is a re-emerging alphavirus that is listed as a NIAID Group III-Category C. CHIKV infection results in debilitating arthralgia in infected individuals. The recent re-emergence of CHIKV has been associated with the acquired ability of the virus to replicate in a more widely spread mosquito vector as well as increased mortality in the elderly and newborn populations. Although several countries are at risk, including the United States, no approved therapeutics or vaccines for CHIKV exist yet.
Role: Developmental Research Project (DP007) PI

OVERLAP No overlap

OTHER SUPPORT - Mark Suto

Research Support

Active

BAO# 16XS124, Suto (PI) 04/1/2016 – 3/31/2020 No Task Orders
Leidos (NCI)

Chemical Biology Consortium — Collaborative Drug Discovery Partnership with NCI. As a specialized center SR will contribute to all aspects of the programs identified by the NCI including screening, medicinal chemistry in vivo studies through final drug development.

1U19AI109680, Whitley (PI) 03/01/2014 – 02/28/2019 (b)(4)
NIAID $1,646,665

Antiviral Drug Discovery and Development Center. The goals of this NIAID program are the development of antiviral drugs for the treatment of emerging and reemerging infections. Specifically, the focus will be on flaviviruses, alphaviruses, corona viruses and influenza. The goal is to identify compounds working through mechanisms that affect viral replication and develop these leads in a translational manner to new human therapeutics. Role: Co-PI Medicinal Chemistry Core

1R01CA175012-01A, Murphy-Ullrich (PI) 08/1/2014 – 07/31/2019 (b)(4)
UAB (NCI) $143,402

This proposal will combine mechanistic studies with drug discovery efforts to achieve our goal of identifying an orally active lead compound for treatment of Multiple Myeloma. We will further determine the role of the TSP1-TGF-β pathway in MM through use of immune competent and TSP1 null models, by comparison of lead compounds to global TGF-β inhibitors. Role: Co-investigator

(b)(4) Suto, PI 05/01/2015 – 04/30/2017
(b)(4) $76,909

The goal is to develop a primary and backup series of small molecules that could be further developed for use in the treatment of ALS. We will identify a primary lead series that is novel, orally bioavailable, and has CNS penetration using a directed medicinal chemistry effort focused on a current lead compound. The best candidates will be evaluated in vivo in the SOD-1 G93A ALS animal model to assess efficacy and target engagement.

Suto (PI) 9/14/2015 – 9/13/2020 (b)(4)
(b)(4) $1,187,439

Goal: The goal is to identify novel read-through drugs for the treatment of cystic fibrosis. Initially, a large high-throughput screen will be run and the compounds further evaluated in several mechanistic assays. Lead optimization and profiling of the compounds will be initiated to identify a preclinical candidate.

U54TR001368-01 (Kimberly) 9/1/15-8/31/20 (b)(4)
NIH/NCATS $6,324,075 (UL1, KL2, TL1)
UAB Center for Clinical and Translational Science (CCTS)
Role: Co-Investigator

Goal: The UAB CCTS will enhance human health by driving scientific discovery and dialogue across the bench, bedside and community continuum. The CCTS support this overall mission in a highly integrative network of relationships. Success in creating such an environment is dependent upon success in achieving five strategic priorities: 1) enhancing research infrastructure; 2) promoting investigator education, training and development; 3) accelerating discovery across the T1 interface; 4) expanding value-added partnerships; and 5) building sustainability.

R01 DK059600-12 Agarwal (PI) 4/01/2016 – 3/31/2017 (b)(4)
UAB/NIH $135,594

Goal: The goal is to identify compounds that up-regulate heme-oxygenase 1 and evaluate them in a model of kidney disease. The compounds were identified using a high-throughput screening strategy.

Role: Co-Investigator

E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. OVERALL CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

As stated in the year 2 update, for Core B, the screening collection was reduced to ~222K for WNV and FLUV by omitting the Chembridge Set 3 to stay within budget. The collection will be expanded back to ~315K compounds by re-including the Chembridge Set 3 for the ZIKV HTS campaign since adequate supplemental funds were awarded.

Core C was designed to escalate activities in Years 3 and 4 in order to accommodate hit-to-lead phases in all four projects. However, the activities of Core C is dependent of Core B finishing the HTS screens and implementation of SAR assays. Core C successfully initiated hit confirmation and hit to lead chemistry from HTS mass screen data finished in Year 2. However, HTS screens on WNV and Influenza were completed in second quarter of Year 3 and activities of Core C on these specific projects are dependent upon the completion of the high throughput screens for these viruses. We have recently analyzed HTS data on WNV screen and prioritized chemical series of interest. Fresh commercial samples have been ordered and analyzed for their purity and integrity. Most of the hits were submitted to the biology laboratory for reconfirmation. As we receive the data, we will prioritize which hits to initiate medicinal chemistry. Similarly, the Influenza project HTS hits reconfirmation was recently completed using MDCK cells and the data is being analyzed. Medicinal chemistry efforts will soon start in Year 4 on the hits from these two screens in addition to ongoing efforts on six hit to lead compounds and two lead optimization compounds for other viruses.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. OVERALL SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

File(s) uploaded:
G1 Umbrella Whitley.pdf
G1 Project 4 Whitley.pdf
G1 Core A Whitley.pdf
G1 Project 1 Nelson.pdf
G1 Project 3 Streblow.pdf
G1 Project 2 (b)(6); (b)(3):7 .pdf
G1 Core C Pathak.pdf
G1 Core B (b)(6); (b)(3):7 pdf

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave. South BIRMINGHAM AL 35294

Oregon Health & Science University	096997515	OR-003	3181 SW Sam Jackson Park Rd. Portland OR 97239
Vanderbilt University	004413456	TN-005	3319 West End Avenue Suite 100 Nashville TN 37203
THe University of North Carolina at Chapel Hill	608195277	NC-004	Administrative Office Bldg.Suiste 2200 104 Airport Rd, CB 1350 Chapel Hill NC 27599
University of Colorado at Denver	041096314	CO-006	1300 E. 17th Place, Room W1126 Anschutz Medical Campus Bldg 500 Denver CO 80045
Portland VA Research Foundation	827052887	OR-01	3710 SW US Veterans Hospital Rd Portland OR 97239
Southern Research Institute	006900526	AL-006	2000 Ninth Avenue South Birmingham AL 35205
Washington University	068552207	MO-001	Campus Box 8051 660 S. Euclid St. Louis MO 63110
UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave South BIRMINGHAM AL 352331806
UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave. South BIRMINGHAM AL 35294
Oregon Health & Science University	096997515	OR-003	3181 SW Sam Jackson Park Rd. Portland OR 97239
Vanderbilt University	004413456	TN-005	3319 West End Avenue Suite 100 Nashville TN 37203
THe University of North Carolina at Chapel Hill	608195277	NC-004	Administrative Office Bldg.Suiste 2200 104 Airport Rd, CB 1350 Chapel Hill NC 27599
University of Colorado at Denver	041096314	CO-006	1300 E. 17th Place, Room W1126 Anschutz Medical Campus Bldg 500 Denver CO 80045
Southern Research Institute	006900526	AL-006	2000 Ninth Avenue South Birmingham AL 35205
Washington University	068552207	MO-001	Campus Box 8051 660 S. Euclid St. Louis MO 63110

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Not Applicable

G.1 Umbrella – Whitley

1. Product Development

Not Applicable

2. Biocontainment/Security:

Project 1:

1. Project title: Identification and characterization of anti-flaviviral compounds
2. Project leader: Jay Nelson
3. Collaborators: Alec Hirsch, Jessica Smith (OHSU); Michael Diamond (Washington Univ)
4. BSL laboratory employed: BSL3 (at both OHSU and Washington University)
5. Pathogens evaluated: WNV, DENV, ZIKV

Project 2:

1. Project Title: Inhibitors of coronavirus fidelity and cap methylation as broadly applicable therapeutics
2. Project Leader (b)(6); (b)(3):7 U.S.C. § 8401 MR (Vanderbilt),
3. Collaborator(s): Baric, RS (UNC)
4. BSL Laboratory Employed: BSL3 (Vanderbilt) BSL3/ABSL3 (UNC)
5. Pathogen(s) Evaluated: Both Vanderbilt and UNC: SARS-CoV, MERS-CoV. Both Vanderbilt and UNC Select Agent Certified

Project 3

1. Project title: Novel therapeutic strategies targeting re-emerging alphaviruses
2. Project Leader: Daniel Streblow (OHSU)
3. Project Collaborators: (b)(6); (b)(3):7 U.S.C. § 8401 (UNC), Victor DeFilippis (OHSU) and Thomas Morrison (U Colorado Denver)
4. Labs at the following sites were used:
    a. Vaccine & Gene Therapy Institute/Oregon Health & Science University.
        i. BSL-3/VGTI rm2215A, Small Animal ABSL-3/VGTI, Nonhuman Primate ABSL-3 Building/ONPRC
        ii. Pathogen: Chikungunya Virus
    b. University of North Carolina-Chapel Hill
        i. (b)(3):7 U.S.C. § 8401
        ii. Pathogen: Chikungunya virus, Venezuelan equine encephalitis virus
    c. University of Colorado-Denver.
        i. BSL-3/UCD Anschutz Medical Campus, Small Animal ABSL-3/UCD Anschutz Medical Campus
        ii. Pathogen: Chikungunya Virus

Core B utilized the BSL3 facility at Southern Research for the following projects:

Project number: 1.2
Project Title: Identification and Development of Anti-Flavivirus Lead Drug Candidates
Project Leaders: Michael Diamond

Collaborators:
BSL Laboratory Employed: Southern Research Institute (for HTS activities)
Pathogens Evaluated: West Nile (NY99)

Project number: 2
Project Title: Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics
Project Leaders: (b)(6); (b)(3):7 U.S.C. § 8401, Ralph Baric
Collaborators:
BSL Laboratory Employed: Southern Research Institute (for HTS activities)
Pathogens Evaluated: SARS Toronto-2 and SARS Urbani/Nanoluc Clone

Project number: 3
Project Title: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
Project Leaders: Daniel Streblow, (b)(6); (b)(3):7 U.S.C. § 8401
Collaborators:
BSL Laboratory Employed: Southern Research Institute (for HTS activities)
Pathogen Evaluated: CHIKV Sri Lanka strain

3. Follow-on Funding
Not applicable

G1 Project 4 – Whitley

1. Significant Changes in Specific Aims

Not Applicable.

2. Significance of the Work

Influenza A viruses continue to emerge from the aquatic avian reservoir and cause seasonal epidemics and infrequent pandemics. Recent experimental evidence by our group and others support the development of novel antivirals targeting the influenza polymerase function. The discovery of molecules that inhibit influenza virus RNA replication is essential to complement the existing drug arsenal, which is proving less effective due to the increasing incidence of mutational resistance.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

Not Applicable.

G1 Core A Special Requirements

The Admin Core has served all Projects and Cores by the following activities:

- Coordinated preparation and execution of year three annual subaward amendments for all sites.
- Processed payments to sites following submission of invoices.
- Planned and hosted the third annual AD3C meeting in which all personnel got to meet each other in person, thereby facilitating further interactions between projects and cores.
- Arranged for NIAID Program Officers to join said annual meeting so they were able to receive an in-depth update on the CETR's progress
- Communicated updates related to NIH grant issues, particularly those that impact the areas being investigated by AD3C investigators
- Solicited applications and project ideas for supplemental funds available for research related to Zika virus and general supplemental research proposals; the latter were reviewed by at least three scientists; the External Advisory Board ultimately chose the two projects that were forwarded to NIAID for consideration of funding.
- Provided feedback from the External Advisory Board reviews to the Projects and Cores.

G1 Project 1 – Nelson

1. Signifance Changes in Specific Aims

Not Applicable.

2. Significance of the Work

The flaviviruses are associated with significant morbidity, mortality, and economic burden throughout world. Nevertheless, no specific anti-viral therapies for disease associated with these viruses are currently available. This project is designed to identify and develop small molecule anti-viral therapeutics against two medically important flaviviruses--dengue virus and West Nile virus. Furthermore, we will emphasize the development of drugs that show activity against multiple flaviviruses, and possibly other virus families as well.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

Not Applicable.

G1 Project 3 – Streblow

1. Significant Changes in Specific Aims

Not Applicable.

2. Significance of the Work

Our Program aims to develop novel antiviral agents to emerging human viral pathogens. This Project will develop broad-spectrum nucleoside/nucleotide inhibitors against Alphaviruses with a focus on Chikungunya virus and Venezuelan Equine Encephalitis virus, both of which are human pathogens that cause severe disease and are associated with mortality and with no currently FDA approved vaccine or therapeutics for treatment.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

1. **THF-ΔIRF-3:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These cells constitutively express the reverse Tet-transactivator via lentivector (Clontech # 631069); not relevant for this study but just FYI. The IRF3 gene sequence has been disrupted using the CRISPR/Cas9 system (AddGene vector # 49535). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 per vial and can be brought up directly into a T75 + 14mL media. Once confluent they can be split 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell line constructed by Dr. DeFilippis.
2. **THF-ΔIFIT1, THF-ΔIFIT2, THF-ΔSTING, THF-ΔIPS1, THF-ΔSTAT1:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These are also stably transduced with a firefly luciferase-coding region under the control of the interferon responsive element using a lentivector obtained from System Biosciences. Individual cell lines were constructed in which the protein coding regions for IFIT1, IFIT2, STING, IPS1, or STAT1 were disrupted using the CRISPR/Cas9 system (AddGene vector # 52961). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 per vial and can be brought up directly into a T75 + 14mL media. Once confluent they can be split 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell lines constructed by Dr. DeFilippis.
3. **CHIKV Caribbean Strain Infectious Clone:** $CHIKV_{99659}$ was recently isolated from the British Virgin Islands in December of 2013. A low-passage stock of this strain was provided to the members of the Alphavirus group from Dr. Michael Diamond (Project 2). The [redacted] lab, in collaboration with Dr. Nathanial Moorman at UNC, has sequenced the isolate and constructed an infectious clone of the virus.
4. **$CHIKV_{181/25}$ Strains Expressing nano-Luciferase (nLuc):** Into the infectious clone of $CHIKV_{181/25}$ was introduced an in-frame nLuc reporter gene. Two different viruses were constructed by the Heise Lab: pTH1.2 (NSP-3nLuc) and pTH2.1 (Capsid-nLuc), which will be utilized by SR for cherry-pick validation screens and for mechanism of action studies.
5. **$CHIKV_{AF15561}$ strain expressing mKate:** An in-frame mKate reporter gene was cloned into the infectious clone of the pathogenic parental virus of $CHIKV_{181/25}$ ($CHIKV_{AF15561}$). Constructed by Dr. Morrison's group.
6. **G10:** A novel small molecule (4-(2-chloro-6-fluorobenzyl)-N-(furan-2-ylmethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazine-6-carboxamide) capable of blocking Alphavirus replication by activating STING-dependent activity in human cells was characterized and described by Dr. DeFilippis.

G1 Project 2 – (b)(6); (b)(3):7 U.S.C. § 8401

1. Signifance Changes in Specific Aims

Not Applicable.

2. Significance of the Work

In this Project we will use extensive small molecule libraries and a sensitive high-throughput in vitro screening assay to identify inhibitors of SARS-CoV replication fidelity and RNA capping that will lead to profound in vivo attenuation, and potentially represent broadly-efficacious inhibitors of endemic and emerging CoVs.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

Not Applicable.

G1 Core Specific Information Core C

A detailed summary of Core C interactions with all four individual Research Projects (1-4) and Core B with respect to the activities as described in original grant proposal are as below.

1. **Research Project 1 (Flaviviruses):**

DENV-: Continuing from Year 2 efforts on hits from 300K+ AD3C screen in which 55 hits were selected as active compounds. These 55 compounds were further triaged using PAINS (pan-assay inhibitors) filtering and removed any other promiscuous, cytotoxic, or undesirable compounds. These analyses left 11 compounds remaining as potential lead compounds which were sent for re-confirmation and tested for antiviral potency (EC_{90}) and efficacy (VTR) at the Research Project-1 site. These compounds were also screened in a new SAR MB assay. After combining both sets of data, three hits; SRI-35847 [EC_{90} = 2.9 μM, CC_{50} >30μM, VTR = 3.5 logs]; SRI-33361 [EC_{90} = 0.9 μM, CC_{50} >5μM, VTR = 3.3 logs] and SRI-36204 [EC_{90} = 0.5 μM, CC_{50} >30 μM, VTR = 1 log] were selected for initial hit to lead chemistry phase. ADME properties on these compounds were also evaluated:SRI-35847 [mouse microsomal stability: t½ = 4 min and solubility = 13 μM]; SRI-33361 [mouse microsomal stability: t½ = 1 min and solubility = 9 μM] and SRI-36204 [mouse microsomal stability: t½ = 59 min and solubility = 2 μM]. A small set of analogs of SRI-35847 were synthesized to determine if this hit warrants further structural development for improved antiviral potency and efficacy. Total of 25 diverse substituted analogs of the primary core were synthesized and evaluated for antiviral activity by Research Project-1 as well as in MB assay. Most of these compounds showed no improvement in efficacy (VTR in logs) as compared to initial hit SRI-35847 and some compounds completely lost antiviral activity. This series was abandoned and chemists were moved to SRI-36965 for hit to lead chemistry. A total of 15 specific compounds were synthesized around the core structure of SRI-36965 but no clear SAR was obtained; hence, further work in this series is on hold and a new plan is being discussed.

Recently, Core C started medicinal chemistry on SRI-33361 which showed excellent antiviral potency and efficacy [EC_{90} = 0.9 μM and VTR = 3.3 logs], but possessed cytotoxicity (CC_{50} >5μM). Chemists identified some functional groups in the molecule that may be responsible for cytotoxicity and thus far, 15 specific analogs were designed and synthesized. These compounds were recently submitted to Research Project-1 for potency (EC_{90}) and VTR assays as well as to Assay Development Core (Core B) for MB assay (data pending). After evaluating the viral activity data and ADME properties [aqueous solubility, log D, and mouse and human microsomal stability], we will evaluate the potential of the hit as to whether to initiate the design of new molecules and push forward in a hit to lead chemistry plan.

WNV: In this project, Core C continued on with the Year 2 results on two re-confirmed hit SRI-33625 and SRI-22003 (MLPCN screen analysis) which showed significant antiviral activity, i.e. EC_{90} = 0.1 μM and EC_{90} = 3.0 μM, respectively with CC_{50} >25 μM. However, after testing in the antiviral efficacy assay, these compounds only showed moderate efficacy (VTR < 2 logs). These two hits were also not very attractive structurally either and thus, Core C decided not to pursue hit-to-lead chemistry on these compounds. While waiting on the HTS mass screen for WNV to be completed by Core B, Core C collected a set of 32 potent reconfirmed HTS hits from SARS, VEEV, CHIKV and DENV screens and supplied these compounds to Research Project-1 to test for antiviral activity against WNV using HEK293 cells. None of these compounds showed any significant antiviral activity.

After the completion of HTS screening campaign on 197K+ compounds in third quarter of Year 3, dose response data was analyzed by Core C. The HTS screens were performed in the following two ways: ***Screen-A***) Targeted mechanism: Inhibition 2'-O-Methyltransferase A, and ***Screen-B***) Secondary mechanism: Direct antiviral effect. A total of 30 compounds were active in Screen-A and were also non-cytotoxic, and 130 compounds were active in Screen B and also non-cytotoxic. A total of 130 hits were analyzed in PAINS filtration. From these active sets, one duplicate compound was found and seven compounds were filtered through PAINS filtration. Clustering analysis was performed on 121 compounds in which 84 singletons and 15 clusters were found. Based on activity and structural desirability, 22 compounds (10 from Screen A and 12 from Screen B) were selected for re-confirmation studies at Research Project-1 sites. Only 21 compounds were commercially available and fresh samples were purchased. Re-synthesis of one the remaining compound will be investigated by Core C. The fresh samples acquired commercially were tested for purity (HPLC) and integrity (HR-MS and ^{1}HNMR). From these 21 compounds, two compounds showed HPLC purity <90% which are now being purificatied by preparative HPLC. The remaining 19 pure samples (>95%) were submitted for

reconfirmation in the assay at the Research Project-1 sites, being evaluated for antiviral potency (EC_{90}) and efficacy (VTR). ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability will also be evaluated on all reconfirmed hits with VTR >2 logs. These results will help to prioritize hit compounds before initiating medicinal chemistry on two selected hits for generating leads for optimization in Year 4 of the project.

2. Research Project 2 (Coronaviruses)

In continuation with chemistry efforts from Year 2 by Core C for this project, hit-to-lead chemistry efforts were performed on three HTS re-confirmed hits: SRI-35293 [ADR EC_{90} = 2.5 μM, NL EC_{50} = 6.8 μM, CC_{50} > 30 μM, VTR > 3 logs], SRI-33684 [ADR EC_{90} = 1.7 μM, NL EC_{50} = 2.8 μM, CC_{50} > 30 μM, VTR = 2 logs], and SRI-33911 [ADR EC_{90} <1 μM, NL EC_{50} = 0.9-4.0 μM, CC_{50} >30 μM, VTR >2 logs]. ADME data on these three hit compounds indicated low solubility and poor mouse microsome stability.

Initial hit-to-lead chemistry was performed on SRI-33684. This compound showed reasonable potency and efficacy [ADR EC_{90} = 1.7 μM, NL EC_{50} = 2.8 μM, CC_{50} > 30 μM, VTR = 2 logs]. SRI-33684 also possessed reasonable ADME properties, decent mouse microsomal stability (t½ = 54 min) but had poor solubility (2 μM). A key structural issue with this hit was the presence of a nitrophenyl moiety that potentially can lead to toxicity *in vivo*. An attempt to substitute this nitrophenyl moiety with other suitable ring systems and bioisosteres of a nitro group was carried out. Approximately 30 analogs were designed and synthesized. These newly synthesized compounds were screened in the SARS virus SAR-NL assay at Core B, but most of the analogs were found inactive.. It was concluded that since activity was lost upon removing the nitro group, this moiety perhaps was contributing to the observed activity.Thus, this chemical series was abandoned for further hit to lead chemistry.

Another hit-to-lead candidate, SRI-35293 possesses an internal triple bond which also can be a liability due to its potential reactivity property. Hence, 28 unique bicyclic structures replacing this triple bond in the molecule were designed and synthesized. A benzothiazole analog, SRI-35804 [EC_{50} = 12.8 μM, CC_{50} > 30 μM], showed activity in SAR-NL assay. However, a fragment of this SRI-35804, showed promising activity [SRI-35742 , EC_{90} = 6.7 μM, CC_{50} > 30 μM] against SARS virus in NL assay. A hit to lead campaign started on hit SRI-35742 and 31 new analogs were designed and synthesized. These new analogs were screened in the SAR-NL assay against SARS virus which resulted in a lead compound, SRI-36565, with an EC_{90} = 0.8 μM and CC_{50} > 30 μM. SRI-33665 is not very stable in mouse liver microsomes (t½ = 12 min) and its solubility is 8 μM. This compound is under evaluation in Research Project 2 site for its potency in a CPE assay and efficacy in a VTR assay using VeroE6 cells. Once these results are obtained, a hit-to-lead campaign will begin to improve ADME properties, potency and efficacy and target EC_{50} <500 nM (EC_{90} <1 μM), a CC_{50} > 20 μM, microsomal stability (mouse) of a $t_{1/2}$ > 60 min and solubility ≥10 μM. A fresh sample of the HTS re-confirmed hit, SRI-33911, by the NL assay was supplied to Research Project-2 site for its potency in a CPE assay and efficacy in a VTR assay using VeroE6 cells. When these results are receiveded, a hit to lead campaign will start targeting improved ADME properties, potency and efficacy in the ranges described above.

3. Research Project 3 (Alphaviruses)

VEEV: Core C continued chemistry from Year 2 on a MLPCN re-confirmed hit, SRI-33394, which showed excellent antiviral activity in a Normal Human Dermal Fibroblasts (NHDF) cell line against VEEV (EC_{90} = 0.7 μM, CC_{50} > 30 μM and VTR = 6.5 logs). However, this compound had very low mouse microsome stability (t½ = 2.1 min). This instability may likely be attributed to the presence of a thiourea and furan functionality in the molecule. Approximately 70 rationally designed analogs of SRI-33394 were synthesized and based on the antiviral data of these compounds, the sulfur atom of the thiourea functionality deemed to be very important for activity. An analog,SRI-34339, a thiourea analog, showed enhanced antiviral potency and efficacy (EC_{90} = 0.01 μM, CC_{50} > 30 μM and VTR = 8.3 logs) but still had a t½ = 1.3 min in mouse liver microsomes. The medicinal chemistry approach then turned to mimetics of a thiourea functionality as well as replacing the ethyldimethylamine side chain. It was anticipated that these new analogs would have better microsomal mouse stability and decent antiviral activity. At least 40 analogs of SRI-34339 were designed, synthesized and submitted to Research Project-3 to be tested in the antiviral assay. One compound from this series, SRI-34339, exhibited good antiviral potency and efficacy, and reasonable ADME properties. Overall, the antiviral

activity and ADME results were variable for these new thiazole analogs (replacing the thiourea with thiazole in SRI-34339 as well as ethyldimethylamine side chain). The potency for most new analogs improved (>0.8 μM) and the microsomal stability improved at least 30-fold. However, the new analogs showed toxicity at < 10 μM in a 72h cell viability assay (NHDF cell line). Thus, this series is currently on hold until a collection of five-six specifically designed analogs can be synthesized and tested to evaluate the emergence of this observed cytoxicity.

After completion of the HTS screening campaign for VEEV on 197K+ compounds in first quarter of Year 3, dose response data was analyzed by Core C. A total of 813 hits were analyzed in PAINS filtration and by clustering analysis. From the active set, 48 compounds were filtered through PAINS filtration. Clustering analysis was done on 765 compounds in which 334 singletons and 470 clusters were identified. From these 470 clusters, 24 clusters were found with > 5 members. The data on these compounds were sorted by EC_{50} and SI, and compounds with EC_{50} < 10 μM and SI > 20 were selected for further investigation. Some compounds were also filtered by visual inspection of structures possessing unwanted functional groups, and core structure uniqueness and commercial availability. A total of 17 compounds were selected and repurchased for re-confirmation in the antiviral assay. The fresh samples that were commercially obtained were tested for their purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before submitting to the reconfirmation assay in Research Project-3 site. A total of 11 hit compounds from the HTS screen re-confirmed in the CPE-based antiviral assay. These compounds were then tested in the VTR assay. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, were evaluated on all confirmed hits. These results helped to prioritize hits and select for initiating medicinal chemistry. SRI-36427 was selected based on its antiviral and VTR activity (EC_{90} = 0.25 μM; CC_{50} > 50 μM; VTR = 10 logs at 12.5 μM with no virus at 25 μM) SRI-36427 was very unstable in mouse liver microsomes (t½ = 2 min) but had reasonable solubility (25 μM). Thirty diverse analogs of SRI-36427 have been synthesized and tested, exhibiting a range of potency and efficacy. Medical chemistry studies of scaffold hopping are underway in an effort to identify a structurally unique lead. Lead generation efforts of hit SRI-36427 is early stages. Medicinal chemistry will continue with a goal to identify a lead compound with improved mouse microsomal stability (t½ > 60 min) and solubility (> 10 μM) which can then be optimized with the goal of identifying a compounds to be tested in animals for antiviral efficacy and potency.

CHIKV: Core C also continued with medicinal chemistry efforts from Year 2 of the project to develop a lead compound from a previous MLPCN VEEV screen hit, SRI-33366, which showed EC_{90} = 3.2 μM; CC_{50} > 50 μM and VTR = 1.7 logs against CHIKV. An analog of SRI-33366 was identified This analog, SRI-34963, showed good antiviral activity (EC_{90} = 1.5 μM, CC_{50} > 25 μM, VTR = 3.9 logs) and reasonable ADME properties (mouse microsomal stability: t ½ = 11 min; solubility 20 μM). At least 120 analogs of SRI-34963 were synthesized and tested for antiviral potency and efficacy using the NHDF cell line in Research Project-3 lab. Several of the compounds showed excellent antiviral potency and efficacy with reasonable ADME properties. Five compounds [SRI-34963, SRI-36172, SRI-36282, SRI-36498 and SRI-36778] were selected for *in vivo* pharmacokinetic (PK) study using *iv* administration route in mice with a dose of 1 mg/kg. From this study, SRI-36498 showed encouraging PK results [AUC_{last} = 192 hr.ng/ml; $t_{1/2}$ terminal phase of elimination = 1.5 h; body clearance (Cl) = 5162 mL/hr/kg; and volume of distribution (Vss) = 5649 mL/kg). Gram scale synthesis of SRI-36498 was performed and a PK study via oral (5 and 10 mg/kg), subcutaneous (5 mg/kg), intraperitoneal (5 mg/kg) and intravenous (1mg/kg) administration in male C57BL/6 mice is underway.

At least 35 selected analogs of SRI-34963 and its reverse amide SRI-36498 were designed and are currently being synthesized in an effort to identify improved analogs of SRI-36498 for testing in animals during Year- 4 of the project.

The structural biology group has performed some target identification and binding studies on SRI-34963 which are based on preliminary data that has been generated from virus resistant studies done at Research Project-3 site. Different truncated proteins of CHIKV nsP3 (i.e., 1-160, 1-180, 1-246 and 1-494) were purified by Ni-NTA and size-exclusion chromatography. Except for nsP3 (1-160), the results indicated that other truncated nsP3 proteins have a degradation problem after purification. The binding of compound, SRI-34963, to nsP3 proteins was measured by an Octet system using a Ni-NTA biosensor. The binding affinity (Kd) was at a single digit micromolar level showing not much difference in the binding affinity of SRI-34963 among different truncated proteins. This may suggest that nsP3 (1-160) is the minimal domain for compound binding. The X-ray diffraction of the crystals of nsP3 (1-160) grown in sodium citrate were tested with a home-source X-ray beam with a resolution of 2.3Å. In future studies, the binding of the compound to nsP3 (1-160) will be tested

using isothermal calorimetry (ITC) and the results will be used to compare and confirm the binding of the compound to nsP3 protein. When nsP3 is validated as the target of SRI-34963, the compound will be soaked into nsP3 crystals or cocrystallized with nsP3(1-160) to obtain the atomic information of the binding site of the compound at nsP3.

After the completion of the HTS screening campaign for CHIKV on 197K+ compounds in the first quarter of Year 3, dose response data was analyzed by Core C. A total of 2194 hits underwent PAINS filtration and clustering analysis. 29 duplicate compounds were identified from the active set of compounds and 138 compounds were triaged through PAINS filtration. Clustering analysis was performed on 2027 compounds in which 695 singletons and 1083 clusters were found. From 1083 clusters, there were 46 clusters with more than six members. The data on these compounds was sorted by EC_{50} values and SI. Compounds with an EC_{50} < 10 μM and SI > 20 were selected. Some compounds were also filtered by visual inspection of structures possessing unwanted functional groups, core structure uniqueness and commercial availability. Finally, 22 compounds were selected and repurchased for re-confirmation in the antiviral assay. Fresh solid samples were tested for purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before submitting for testing in the reconfirmation assay in Research Project-3 laboratory. A total of 11 Hits from the HTS screen that were reconfirmed using the CPE-based antiviral assay were then tested in the VTR assay. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, were evaluated on all confirmed hits. These results help to prioritize hits before initiating medicinal chemistry on two of the best hits, SRI-36767 [EC_{90} = 0.1 μM; CC_{50} > 127 μM; VTR = 6.9 logs at 6.25 μM] and SRI-36768 [EC_{90} = 0.2 μM; CC_{50} > 112 μM; VTR = 7.6 logs at 0.78 μM]. ADME properties were also evaluated on SRI-36767 (mouse microsomal stability: t½ = 2 min; solubility = 58 μM) and SRI-36768 (mouse microsomal stability: t½ = 3 min; solubility = 2 μM). Synthesis of analogs of SRI-36767 was initially planned but due to synthetic challenges of the targeted anlaogs and the chemical instability, this effort was suspended. However, medicinal chemistry is planned for analogs of SRI-36768 with the goal of identifying a lead compound that has improved mouse microsomal stability (t½ >60 min) and solubility (>10 μM) and warrants further testing in an animal model.

In an attempt to identify some cross virus active molecules within the same virus family, the reconfirmed hits from VEEV and CHIKV hits were tested in the different virus assays. From this testing, two compounds were identified that showed potential activity versus both viruses. These two compounds, SRI-36426 and SRI-36768, are considered dual inhibitors and were further tested in both the CPE and VTR assays against both viruses. The results were for SRI-36426 were: VEEV [EC_{90} = 0.72 μM; CC_{50} > 50 μM; VTR = 6 logs at 12.5 μM and no virus at 25 μM]; CHIKV [EC_{90} = 1.2 μM; CC_{50} > 50 μM; VTR = 3 logs at 12.5 μM] and the results for SRI-36768 were: VEEV [EC_{90} = 0.7 μM; CC_{50} > 30 μM; VTR = 6 logs at 10 μM]; CHIKV [EC_{90} = 0.2 μM; CC_{50} > 30 μM; VTR = 7.6 logs at 0.78 μM]. The ADME properties for each of these compounds were also determined: [SRI-36426: mouse microsomal stability: t½ = 5 min; solubility = 26 μM and SRI-36768: mouse microsomal stability: t½ = 3 min; solubility = 2 μM]. Twenty-four new analogs of SRI-36426 have been designed and synthesized recently and submitted to Core B to screen in a combination assay of antiviral effect (EC_{50} and VTR) as well as for testing at the Research Project-3 site. So far, few analogs have shown an improvement in imicrosomal stability and solubility while maintaining acceptable potency and efficacy. Hit to lead development is in a very early stage for this chemical series and will continue in Year 4 of this project. However, structural changes to SRI-36768 with the goal to improve the compound's ADME properties will be synthesized in Year 4 of the project. Based on the activity and ADME profiles from each series, one lead compound will be further optimized with the goal of identifying a compound to test in animals.

4. Research Project 4 (Influenza A virus)

Core C chemistry efforts continued on SRI-34993, a compound that was identified in year 3 and showed antiviral activity in two of the strains [H1N1 (EC_{50} = 3.2 μM, in HEK293 cells) and H3N2 (EC_{50} = 2.9 μM, in HEK293 cells)]. Twenty-five commercial analogs of SRI-34993 were purchased and screened against both virus types in MDCK cells. Unfortunately, none of these compounds showed any activity. Hence, the series was not pursued further. While awaiting the completion of the HTS influenza mass screen, Core C collected a set of 32 reconfirmed HTS hits from SARS, VEEV, CHIKV and DENV screens and supplied these compounds to Research Project-4 to test for antiviral activity against H3N2 strain using HEK293 cells. Several compounds showed antiviral activity in HEK293 cells but did not show any antiviral activity against both H3N2 and H1N1

strains when using MDCK cells.

After completion of the HTS screening campaign in the third quarter of Year 3, dose response data was analyzed by Core C. A total of 1196 hits underwent PAINS filtration and clustering analysis.. From the active set of compounds, 16 duplicate compounds were resulted and 94 compounds were selected for PAINS filtration. Clustering analysis was done on 892 compounds from which 420 singletons and 161 clusters were identified with nine clusters having greater than six members. These 892 compounds were also screened for antiviral activity against H1N1 and H3N2 strains at 1.25 µg/mL and 10 µg/mL concentration using MDCK cells. A total of 16 compounds that were active in both strains (> 50% inhibition) with cell viability > 75% were selected. A fresh solid sample of each of these 6 compounds is being acquired which will then be evaluated for purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before tested for antiviral activity in CPE and VTR assays. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, will also be evaluated on all confirmed hits before initiating chemistry on two of the best hits. All of these results will help us prioritize hits for generating leads for medicinal chemistry in Year 4.

G1 Core specific information Core B

Project 1. Flaviviruses

Dengue virus

Aim 1 (accomplished Year 1): A CPE assay employing a dengue viral stock prepared in insect cells and HEK293 host cells was used to screen a total of 304,810 compound samples. Using an activity threshold of inhibition ≥ 26.25% (mean + 3xSD of all data), 2,240 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Forty-five (45) compounds were confirmed and validated as hits with $IC_{50} < 20$ µM and no cytotoxicity. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An immunofluorescence assay measuring viral protein expression in the host cell was developed and is being used to develop SAR for hit-to-lead and lead optimization chemistry efforts.

West Nile Virus

Aim 1 (accomplished Year 3): A CPE assay was constructed to identify inhibitors of the viral 2'-O-Methyltransferase. The 2'-O-MTase activity of flaviviruses promotes viral evasion of the Ifit family of genes, a group of host cell IFN-stimulated immune effector proteins. In order to detect inhibitors of virus 2'-O-MTase activity, the HTS assay was performed using transformed HEK 293 cells that expressed Ifit1 when induced by doxycycline. Such compounds will promote the host cell defense mechanism and reduce CPE. The assay also detected compounds that had a direct anti-viral effect since those compounds reduced CPE independently of Ifit expression. A total of 197,077 compounds were screened using HEK cells treated with doxycycline to induce ifit1 expression. Using a statistical threshold of inhibition ≥ 19.03% (mean + 3xSD of all data), 2997 compounds were identified as active. In order to confirm hits and distinguish potential inhibitors of 2'-O-MTase activity from those with direct anti-viral activity, the compounds were retested at 10 concentrations for inhibition of CPE and direct cytotoxicity effects in HEK cells treated with or without doxycycline (i.e. with or without ifit1 expression). IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Hits were deemed confirmed and valid if they had an $IC_{50} < 75$ µM and no cytotoxic effect. By this criteria, 30 compounds were active only if ifit1 was expressed (i.e. active only in cells treated with doxycycline) and were identified as potential inhibitors of the viral 2'-O-Methyltransferase. An additional 130 compounds were active independent of Ifit expression and identified as those having direct anti-viral effects. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): The CPE assay is currently available to develop SAR for hit-to-lead and lead optimization chemistry efforts but an immunofluorescence assay is being developed for future use.

Project 2. SARS Corona Virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells selected for expression of the SARS CoV receptor (ACE2; angiotensin-converting enzyme 2) were used to screen a total of 305,648 compound samples. Using an activity threshold of inhibition ≥ 80%, 2,492 samples

were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Of these, 307 compounds were confirmed and validated as hits showing IC_{50} < 20 μM and SI (IC_{50}/CC_{50}) > 3. An additional 268 compounds were confirmed and validated as hits showing IC_{50} > 20 μM and SI (IC_{50}/CC_{50}) > 3. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An assay measuring reporter luminescence as an index of virus titer was developed using a recombinant SARS Nanoluc virus produced in the Baric lab. The assay is employed to measure the anti-viral effects of newly synthesized compounds to support development of SAR for hit-to-lead and lead optimization chemistry efforts.

Project 3. Alpha Viruses

Chickungunya virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells were used to screen a total of 197,025 compound samples. Using an activity threshold of inhibition ≥ 50.38% (mean + 3xSD of all data), 2,558 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects using Teleomerized Human Fibroblast (THF) cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. Forty-four (44) hits were confirmed and validated with IC_{50} < 20 μM and SI (IC_{50}/CC_{50}) > 10. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An assay measuring reporter luminescence as an index of virus titer was developed using a recombinant CHIKV Nanoluc virus produced in the (b)(6); (b)(3):7 ab. The assay is employed to measure the anti-viral effects of newly synthesized compounds to support development of SAR for hit-to-lead and lead optimization chemistry efforts.

Venezuelan Equine Encephalitis virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells were used to screen a total of 197,025 compound samples. Using an activity threshold of inhibition ≥ 12.12% (mean + 3xSD of all data), 940 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects using Teleomerized Human Fibroblast (THF) cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. Forty-two (42) hits were confirmed and validated with IC_{50} < 20 μM and SI (IC_{50}/CC_{50}) > 10. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): The CPE assay is currently available to develop SAR for hit-to-lead and lead optimization chemistry efforts. A virus titer reduction assay has also been developed to use in conjunction with the CPE assay data.

Project 4. Influenza A viruses

Aim 1 (accomplished Year 3): An enzyme linked virus inhibitor reporter assay was used for HTS. This assay (described in Lutz et al., J. Virol. Methods 2015, 126: 13-20) utilizes an

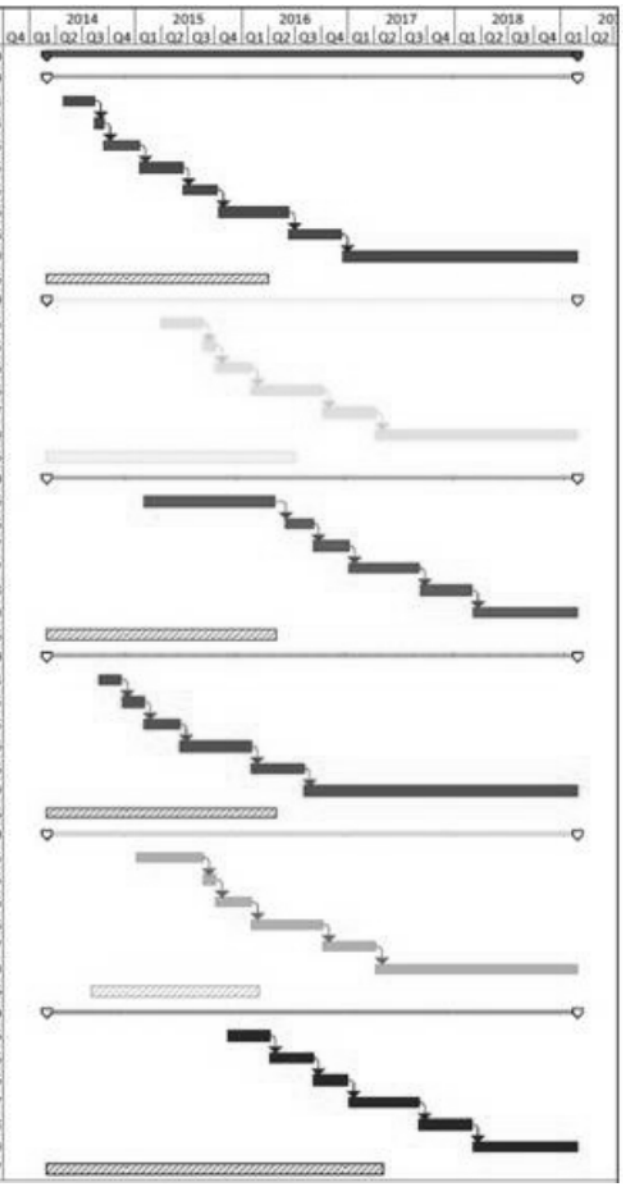
HEK293 cell line engineered to express virus-like negative sense RNA transcripts encoding firefly luciferase flanked by the untranslated regions of influenza A/WSN/33 NP segment. (ELVIRA® Flu A-luc cells). When these cells are infected by influenza A, the virus RdRp transcribes this RNA into mRNA and luciferase protein is produced. Luciferase enzyme activity is then measured as a reporter of virus infection enabling the anti-viral activity of test compounds to be determined by a decrease in luciferase activity. A total of 196,721 unique compounds were screened in HTS. Using an activity threshold of inhibition ≥ 83.45% (mean + 3xSD of all data), 3200 samples were identified as active and retested at 10 concentrations for anti-viral and direct cytotoxicity effects. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. A total of 1197 hits were confirmed and validated showing IC_{50} < 20 μM and SI (IC_{50}/CC_{50}) > 10 in the ELVIRA® Flu A-luc HEK reporter cells. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): The reporter assay used for HTS can also be employed to measure the anti-viral effects of newly synthesized compounds to support development of SAR for hit-to-lead and lead optimization chemistry efforts.

G.1 (G1 Core P[(b)(6); (b)(3):7] pdf)

Timeline of activities

ID	Task Name	Start	Finish
1	**CETR U19**	**Mon 3/3/14**	**Thu 2/28/19**
2	**DEN Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
3	DEN Assay Development	Thu 5/1/14	Thu 8/14/14
4	DEN HTS	Fri 8/15/14	Mon 9/15/14
5	DEN Assay Troubleshooting	Tue 9/16/14	Wed 1/14/15
6	DEN Resume HTS	Thu 1/15/15	Fri 6/12/15
7	DEN Hit Triage	Mon 6/15/15	Fri 10/9/15
8	DEN Lead Series Identification	Mon 10/12/15	Fri 6/10/16
9	DEN Proof of Concept	Mon 6/13/16	Fri 12/9/16
10	DEN Lead Optimization	Mon 12/12/16	Thu 2/28/19
11	DEN Existing Hit Follow Up	Mon 3/3/14	Thu 3/31/16
12	**VEEV Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
13	VEEV Assay Development	Wed 4/1/15	Fri 8/21/15
14	VEEV HTS	Mon 8/24/15	Mon 10/5/15
15	VEEV Hit Triage	Tue 10/6/15	Thu 2/4/16
16	VEEV Lead Series Identification	Fri 2/5/16	Wed 10/5/16
17	VEEV Proof of Concept	Thu 10/6/16	Thu 4/6/17
18	VEEV Lead Optimization	Fri 4/7/17	Thu 2/28/19
19	VEEV Existing Hit Follow Up	Mon 3/3/14	Thu 6/30/16
20	**H1N1 Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
21	H1N1 Assay Development	Mon 2/2/15	Wed 4/20/16
22	H1N1 HTS	Thu 6/2/16	Wed 8/31/16
23	H1N1 Hit Triage	Thu 9/1/16	Mon 1/2/17
24	H1N1 Lead Series Identification	Tue 1/3/17	Fri 9/1/17
25	H1N1 Proof of Concept	Mon 9/4/17	Fri 3/2/18
26	H1N1 Lead Optimization	Mon 3/5/18	Thu 2/28/19
27	H1N1 Existing Hit Follow Up	Mon 3/3/14	Fri 4/29/16
28	**SARS CPE (BSL3) Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
29	SARS CPE (BSL3) Assay Development	Mon 9/1/14	Fri 11/14/14
30	SARS CPE (BSL3) HTS	Mon 11/17/14	Fri 1/30/15
31	SARS CPE (BSL3) Hit Triage	Mon 2/2/15	Wed 6/3/15
32	SARS CPE (BSL3) Lead Series Identification	Thu 6/4/15	Tue 2/2/16
33	SARS CPE (BSL3) Proof of Concept	Wed 2/3/16	Mon 8/1/16
34	SARS CPE (BSL3) Lead Optimization	Tue 8/2/16	Thu 2/28/19
35	SARS CPE (BSL3) Existing Hit Follow Up	Mon 3/3/14	Fri 4/29/16
36	**CHIKV (BSL3) Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
37	CHIKV (BSL3) Assay Development	Mon 1/5/15	Fri 8/21/15
38	CHIKV (BSL3) HTS	Mon 8/24/15	Mon 10/5/15
39	CHIKV (BSL3) Hit Triage	Tue 10/6/15	Thu 2/4/16
40	CHIKV (BSL3) Lead Series Identification	Fri 2/5/16	Wed 10/5/16
41	CHIKV (BSL3) Proof of Concept	Thu 10/6/16	Thu 4/6/17
42	CHIKV (BSL3) Lead Optimization	Fri 4/7/17	Thu 2/28/19
43	CHIKV (BSL3) Existing Hit Follow Up	Fri 8/1/14	Mon 2/29/16
44	**WNV (BSL3) Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
45	WNV (BSL3) Assay Development	Mon 11/16/15	Tue 4/5/16
46	WNV (BSL3) HTS	Wed 4/6/16	Wed 8/31/16
47	WNV (BSL3) Hit Triage	Thu 9/1/16	Fri 12/30/16
48	WNV (BSL3) Lead Series Identification	Mon 1/2/17	Thu 8/31/17
49	WNV (BSL3) Proof of Concept	Fri 9/1/17	Fri 3/2/18
50	WNV (BSL3) Lead Optimization	Mon 3/5/18	Thu 2/28/19
51	WNV (BSL3) Existing Hit Follow Up	Mon 3/3/14	Fri 4/28/17



Composite Application Budget Summary

Categories	Budget Period
Salary, Wages and Fringe Benefits	178,951
Equipment	0
Travel	51,572
Participant/Trainee Support Costs	0
Other Direct Costs (excluding Consortium)	54,091
Consortium Costs	6,875,088
Direct Costs	7,159,702
Indirect Costs	133,769
Total Direct and Indirect Costs	7,293,471

*This application includes at least one component led by an organization that has a DUNS different than the Applicant Organization. The indirect cost calculation for the applicant organization ma not include all allowed Indirect Costs for the first $25K of requested consortium costs and, therefore, may appear less than expected. No action is required from the applicant; NIH will make any appropriate corrections to the budget calculations administratively. The application review will not be affected.

Component Budget Summary

Components	Categories	Budget Period
8274-001 (Admin Core)	Salary, Wages and Fringe Benefits	89,929
	Equipment	0
	Travel	48,572
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	30,155
	Consortium Costs	0
	Direct Costs	168,656
	Indirect Costs	79,268
TOTALS	Total Direct and Indirect Costs	247,924
8280-001 (Core)	Salary, Wages and Fringe Benefits	724,613
	Equipment	0
	Travel	5,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	295,538
	Consortium Costs	0
	Direct Costs	1,025,151
	Indirect Costs	1,303,264
TOTALS	Total Direct and Indirect Costs	2,328,415
8279-002 (Core)	Salary, Wages and Fringe Benefits	245,569
	Equipment	0
	Travel	2,500

	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	374,766
	Consortium Costs	0
	Direct Costs	622,835
	Indirect Costs	497,031
TOTALS	Total Direct and Indirect Costs	1,119,866
8285-001 (Project)	Salary, Wages and Fringe Benefits	230,651
	Equipment	0
	Travel	9,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	116,556
	Consortium Costs	0
	Direct Costs	356,207
	Indirect Costs	420,850
TOTALS	Total Direct and Indirect Costs	777,057
8284-002 (Project)	Salary, Wages and Fringe Benefits	75,885
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	72,065
	Consortium Costs	0
	Direct Costs	147,950
	Indirect Costs	82,112
TOTALS	Total Direct and Indirect Costs	230,062

8283-003 (Project)	Salary, Wages and Fringe Benefits	89,489
	Equipment	0
	Travel	4,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	56,511
	Consortium Costs	0
	Direct Costs	150,000
	Indirect Costs	78,000
TOTALS	Total Direct and Indirect Costs	228,000
8282-004 (Project)	Salary, Wages and Fringe Benefits	144,461
	Equipment	0
	Travel	6,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	153,910
	Consortium Costs	0
	Direct Costs	304,371
	Indirect Costs	158,273
TOTALS	Total Direct and Indirect Costs	462,644
8281-005 (Project)	Salary, Wages and Fringe Benefits	81,265
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	79,096
	Consortium Costs	0

	Direct Costs	163,361
	Indirect Costs	85,765
TOTALS	Total Direct and Indirect Costs	249,126
8278-006 (Project)	Salary, Wages and Fringe Benefits	89,022
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	23,936
	Consortium Costs	0
	Direct Costs	115,958
	Indirect Costs	54,501
TOTALS	Total Direct and Indirect Costs	170,459
8277-007 (Project)	Salary, Wages and Fringe Benefits	157,849
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	10,000
	Other Direct Costs (excluding Consortium)	128,351
	Consortium Costs	0
	Direct Costs	299,200
	Indirect Costs	216,900
TOTALS	Total Direct and Indirect Costs	516,100
8276-008 (Project)	Salary, Wages and Fringe Benefits	200,459
	Equipment	0
	Travel	3,000

	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	52,633
	Consortium Costs	0
	Direct Costs	256,092
	Indirect Costs	148,533
TOTALS	Total Direct and Indirect Costs	404,625
8275-009 (Project)	Salary, Wages and Fringe Benefits	210,394
	Equipment	0
	Travel	6,500
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	120,145
	Consortium Costs	0
	Direct Costs	337,039
	Indirect Costs	222,154
TOTALS	Total Direct and Indirect Costs	559,193
TOTALS		**7,293,471**

Categories Budget Summary

Categories	Components	Budget Period
R&R Budget - Senior/Key Person Funds Requested	8274-001 (Admin Core)	63,320
	8280-001 (Core)	94,278
	8279-002 (Core)	48,270
	8285-001 (Project)	50,616
	8284-002 (Project)	35,436
	8283-003 (Project)	21,628
	8282-004 (Project)	99,583
	8281-005 (Project)	11,175
	8278-006 (Project)	58,396
	8277-007 (Project)	45,859
	8276-008 (Project)	51,782
	8275-009 (Project)	143,664
TOTALS		724,007
R&R Budget - Other Personnel Funds Requested	8274-001 (Admin Core)	26,609
	8280-001 (Core)	630,335
	8279-002 (Core)	197,299
	8285-001 (Project)	180,035
	8284-002 (Project)	40,449
	8283-003 (Project)	67,861
	8282-004 (Project)	44,878
	8281-005 (Project)	70,090

	8278-006 (Project)	30,626
	8277-007 (Project)	111,990
	8276-008 (Project)	148,678
	8275-009 (Project)	66,730
TOTALS		1,615,580
R&R Budget - Section A & B. Total Salary, Wages and Fringe Benefits (A+B)	8274-001 (Admin Core)	89,929
	8280-001 (Core)	724,613
	8279-002 (Core)	245,569
	8285-001 (Project)	230,651
	8284-002 (Project)	75,885
	8283-003 (Project)	89,489
	8282-004 (Project)	144,461
	8281-005 (Project)	81,265
	8278-006 (Project)	89,022
	8277-007 (Project)	157,849
	8276-008 (Project)	200,459
	8275-009 (Project)	210,394
TOTALS		2,339,586
R&R Budget - Section C. Total Equipment	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0

	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		0
R&R Budget - Domestic Travel	8274-001 (Admin Core)	48,572
	8280-001 (Core)	5,000
	8279-002 (Core)	2,500
	8285-001 (Project)	9,000
	8284-002 (Project)	0
	8283-003 (Project)	4,000
	8282-004 (Project)	6,000
	8281-005 (Project)	3,000
	8278-006 (Project)	3,000
	8277-007 (Project)	3,000
	8276-008 (Project)	3,000
	8275-009 (Project)	6,500
TOTALS		93,572
R&R Budget - Foreign Travel	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0

	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		0
R&R Budget - Section D. Total Travel	8274-001 (Admin Core)	48,572
	8280-001 (Core)	5,000
	8279-002 (Core)	2,500
	8285-001 (Project)	9,000
	8284-002 (Project)	0
	8283-003 (Project)	4,000
	8282-004 (Project)	6,000
	8281-005 (Project)	3,000
	8278-006 (Project)	3,000
	8277-007 (Project)	3,000
	8276-008 (Project)	3,000
	8275-009 (Project)	6,500
TOTALS		93,572
R&R Budget - Tuition/Fees/Health Insurance	8274-001 (Admin Core)	0
	8280-001 (Core)	0

	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	10,000
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		10,000
R&R Budget - Stipends	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		0

R&R Budget - Trainee Travel	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		0
R&R Budget - Subsistence	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0

	8275-009 (Project)	0
TOTALS		0
R&R Budget - Other Participants/Trainee Support Costs	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		0
R&R Budget - Section E. Total Participants/Trainee Support Costs	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0

	8277-007 (Project)	10,000
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		10,000
R&R Budget - Materials and Supplies	8274-001 (Admin Core)	750
	8280-001 (Core)	292,288
	8279-002 (Core)	321,486
	8285-001 (Project)	108,556
	8284-002 (Project)	32,065
	8283-003 (Project)	47,011
	8282-004 (Project)	115,489
	8281-005 (Project)	69,596
	8278-006 (Project)	15,910
	8277-007 (Project)	35,440
	8276-008 (Project)	40,633
	8275-009 (Project)	31,545
TOTALS		1,110,769
R&R Budget - Publication Costs	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	2,000

	8281-005 (Project)	2,000
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	2,000
	8275-009 (Project)	2,000
TOTALS		8,000
R&R Budget - Consultant Services	8274-001 (Admin Core)	12,500
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		12,500
R&R Budget - ADP/Computer Services	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0

	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		0
R&R Budget - Subawards/Consortium/Contractual Costs	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		0
R&R Budget - Equipment or Facility Rental User Fees	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0

	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		0
R&R Budget - Alterations and Renovations	8274-001 (Admin Core)	0
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	0
	8281-005 (Project)	0
	8278-006 (Project)	0
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	0
TOTALS		0
R&R Budget - Other Direct Cost 1	8274-001 (Admin Core)	3,155

	8280-001 (Core)	3,250
	8279-002 (Core)	53,280
	8285-001 (Project)	8,000
	8284-002 (Project)	40,000
	8283-003 (Project)	5,000
	8282-004 (Project)	15,000
	8281-005 (Project)	2,500
	8278-006 (Project)	2,776
	8277-007 (Project)	61,342
	8276-008 (Project)	10,000
	8275-009 (Project)	19,600
TOTALS		223,903
R&R Budget - Other Direct Cost 2	8274-001 (Admin Core)	10,500
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	4,500
	8282-004 (Project)	13,000
	8281-005 (Project)	2,000
	8278-006 (Project)	4,250
	8277-007 (Project)	31,569
	8276-008 (Project)	0
	8275-009 (Project)	7,000

TOTALS		72,819
R&R Budget - Other Direct Cost 3	8274-001 (Admin Core)	3,250
	8280-001 (Core)	0
	8279-002 (Core)	0
	8285-001 (Project)	0
	8284-002 (Project)	0
	8283-003 (Project)	0
	8282-004 (Project)	8,421
	8281-005 (Project)	3,000
	8278-006 (Project)	1,000
	8277-007 (Project)	0
	8276-008 (Project)	0
	8275-009 (Project)	60,000
TOTALS		75,671
R&R Budget - Section F. Total Other Direct Cost	8274-001 (Admin Core)	30,155
	8280-001 (Core)	295,538
	8279-002 (Core)	374,766
	8285-001 (Project)	116,556
	8284-002 (Project)	72,065
	8283-003 (Project)	56,511
	8282-004 (Project)	153,910
	8281-005 (Project)	79,096
	8278-006 (Project)	23,936
	8277-007 (Project)	128,351

	8276-008 (Project)	52,633
	8275-009 (Project)	120,145
TOTALS		1,503,662
R&R Budget - Section G. Total Direct Cost (A thru F)	8274-001 (Admin Core)	168,656
	8280-001 (Core)	1,025,151
	8279-002 (Core)	622,835
	8285-001 (Project)	356,207
	8284-002 (Project)	147,950
	8283-003 (Project)	150,000
	8282-004 (Project)	304,371
	8281-005 (Project)	163,361
	8278-006 (Project)	115,958
	8277-007 (Project)	299,200
	8276-008 (Project)	256,092
	8275-009 (Project)	337,039
TOTALS		3,946,820
R&R Budget - Section H. Indirect Costs	8274-001 (Admin Core)	79,268
	8280-001 (Core)	1,303,264
	8279-002 (Core)	497,031
	8285-001 (Project)	420,850
	8284-002 (Project)	82,112
	8283-003 (Project)	78,000
	8282-004 (Project)	158,273
	8281-005 (Project)	85,765

	8278-006 (Project)	54,501
	8277-007 (Project)	216,900
	8276-008 (Project)	148,533
	8275-009 (Project)	222,154
TOTALS		3,346,651
R&R Budget - Section I. Total Direct and Indirect Costs (G +H)	8274-001 (Admin Core)	247,924
	8280-001 (Core)	2,328,415
	8279-002 (Core)	1,119,866
	8285-001 (Project)	777,057
	8284-002 (Project)	230,062
	8283-003 (Project)	228,000
	8282-004 (Project)	462,644
	8281-005 (Project)	249,126
	8278-006 (Project)	170,459
	8277-007 (Project)	516,100
	8276-008 (Project)	404,625
	8275-009 (Project)	559,193
TOTALS		7,293,471

A. COMPONENT COVER PAGE

Project Title: Administrative Core - Core A

Component Project Lead Information:

Whitley, Richard J.

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The Administrative Core of the Antiviral Drug Discovery and Development Center (AD3C) will provide a key role in leadership, communication, coordination and oversight of the projects and cores, and stimulate collaboration and synergy between the projects. Operationally, it is in charge of fiscal and contractual management of the center and will plan and implement activities, such as meetings of the Executive Committee (EC), External Scientific Advisory Board (EAB), and an annual meeting of all projects and cores. In addition, it will manage the inter-institutional cooperative agreements. The core is also responsible for managing the solicitation and review of Supplemental Research Projects applications, such as those for additional product development and support for IND-enabling studies. Finally, the core will facilitate dissemination of progress and discoveries to the public. The broad objectives of the core are thus as follows:

1.Providing programmatic and administrative leadership
a.Make decisions, specifically “go versus no-go” decisions per discussions with the EC
b.Track and encourage research productivity
c.Promote interactions and collaboration between projects and cores, in particular to facilitate overarching synergy to pursue broad-spectrum antivirals
d.Monitor the direction and overall priorities of the Center
e.Directly interface with NIH staff

2.Fiscal and administrative management of the center
a.Finances: oversee expenditures, budget information, fiscal reports
b.Manage contracts and the consortium agreement
c.Establish and monitor compliance with federal and NIH regulations

3.Develop, support and monitor progress of projects
a.Manage projects by having regular conference calls and in-person meetings
b.Organize quarterly project reviews (face to face or teleconference) by the EC
c.Monitor overall Center research quality and progress annually by the EAB
d.Solicit additional regulatory guidance on an as-needed basis
e.Assist with identification and management of intellectual property developed by projects

4.Stimulate collaboration and synergy
a.Identify potential areas or topics of collaborations between projects and cores
b.Provide and facilitate access to resources needed in the projects
c.Ensure that active hits in one project with potential against other viruses are evaluated in other projects
d.Set up a data sharing and project tracking website

5.Facilitate meetings
a.Host monthly conference calls of project teams
b.Organize conference call-based and in-person meetings of the project and core PIs (EC)
c.Organize and implement Annual face to face meeting with all involved personnel, incl the EAB
d.Facilitate consulting and other scientific and professional meetings
e.Attend the annual CETR Program meeting and reverse site visit

6.Manage Supplemental Research Projects applications
a.Solicit proposals
b.Organize scientific and programmatic review of proposals

7.Outreach to the public
a.Set up and maintain a website
b.Write press releases
c.Publish newsletters/ebriefs
d.Help with scientific publications

We firmly believe that a strong Administrative Core will be crucial to the success of the AD3C. We have aggregated projects led by leading virologists in the country and are confident that the Administrative Core will facilitate communication and collaboration between the PIs and the cores. Accomplishing the objectives set out above will ensure focus and synergy among the projects, accelerating the development of new, potentially broad-spectrum therapeutics for (re-)emerging infections of flaviviruses, coronaviruses, alphaviruses and influenza.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: 161123 B2 Admin.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

In the upcoming reporting period, Yr4 of the AD3C grant, the Admin Core plans to continue its leadership in ensuring the operations and activities of AD3C get executed as efficiently as possible. For this, it will focus on the following activities within the listed goals:

1.Providing programmatic and administrative leadership
a.We will continue our monthly conference calls with research updates of the Projects and Cores, with associated minutes and metric-tracking. The bi-weekly Core update meeting will also continue, as this has proven to be a very effective way of communicating action items and status reports for all of the Projects. In addition, small group meetings will continue be set up between the Projects and Cores to cover technical details that warrant a detailed discussion that does not need to include all members of the Executive Committee.
2.Fiscal and administrative management of the center
a.Core staff will continue to work with sites to ensure that they are aware of any federal regulations that are announced and related to the grant, particularly those that apply to sub-recipient awards. Financial accounts and invoice submissions will be tracked closely and budget updates will continue to be provided to sites on a regular basis as effort on the projects continues to advance.
3.Develop, support and monitor progress of projects
a.As mentioned under item 1, the monthly conference calls have proven to be very efficient in monitoring progress in the projects and cores. In addition, like in Year 3, we plan on having a mid-year face to face meeting with the EC members at the International Conference for Antiviral Research (ICAR), to be held in May 2017 in Atlanta, GA.
4.Stimulate collaboration and synergy
a.Cores B and C continue to maintain a database that can be used to share activities of all tested compounds against the various viruses and assays, to quickly identify compounds with broad-spectrum efficacy. In addition, in the monthly conference calls, areas of collaboration and materials of use to multiple projects continue to be routinely identified and offered by the personnel involved. Core C will continue to identify active molecules with an attractive enough profile to warrant testing in multiple virus families.
5.Facilitate meetings
a.The Admin Core will continue to host the monthly teleconference and ad hoc conferences to address specific concerns. In addition it will organize the mid-year EC face-to-face meeting at the ICAR meeting which is being held in Atlanta, GA, Finally, the core staff will organize the fourth annual AD3C meeting with all personnel involved, in Birmingham, AL. We will once again host the External Advisory Board during the annual AD3C meeting, to give them the most up-to-date information about the research progress prior to their evaluation of the program. We will continue to block sufficient time at the annual meeting to accommodate in depth detailed discussions between Project and Core personnel, in addition to high level presentations.
6.Manage Supplemental Research Projects applications
a.The Admin Core will continue to administer the supplemental funding provided to Core B for Zika virus High Throughput Screening
7.Outreach to the public
a.The Admin Core will maintain and update www.uab.edu/ad3c as pertinent.

The major activities of Core A along with their specific objectives are described in bulleted form below, with a short description of the current status. Overall, Core A has met its objectives in Yr3.

1. Providing programmatic and administrative leadership

a. Make decisions, specifically "go versus no-go" decisions per discussions with the EC

We continued the practice of meeting with the EC and additional personnel on a monthly basis, via teleconference, to get an update on each of the projects and their interaction with the cores.

b. Track and encourage research productivity

The teleconferences mentioned above were summarized in minutes distributed back to the AD3C participants. Metrics such as publications and IP applications are actively being tracked; publications are listed on the website.

c. Promote interactions and collaboration between projects and cores

The projects and cores have been collaborating heavily, with interactions facilitated by the Administrative Core as well as initiated by project and core leaders and personnel themselves to discuss ad-hoc technical issues. Together with the Medicinal Chemistry Core, we are testing active lead compounds from one virus family across all the projects in this CETR, to find broad-spectrum therapeutics.

d. Monitor the direction and overall priorities of the center

The EAB met after the annual AD3C meeting and provided a report to the Administrative Core, attached to the overall CETR component of this progress report.

e. Directly interface with NIH staff

We continued to communicate with NIAID program staff on a regular basis and distributed pertinent information to the other research sites; NIAID staff also scheduled the required yearly site visit in conjunction with our annual meeting to provide direct interaction with investigators.

2. Fiscal and administrative management of the center

a. Finances: oversee expenditures, budget information, fiscal reports

The Administrative Core has provided payment and tracking information to sites and provided guidance on current Year 3 budgeting as well as projections for Year 4.

b. Manage contracts and the consortium agreement

The Administrative Core is working on an agreement between Gilead and the Consortium; expected completion before the end of Yr3.

c. Establish and monitor compliance with federal and NIH regulations

The Administrative Core staff continues to maintain a contact list which includes key research, administrative and financial personnel, and is in regular communication with them regarding all AD3C activities and regulatory and financial requirements under the award. The Business Officer continues to work closely with UAB's Office of Sponsored Program and Grant Accounting as well as with similar offices in the participating institutions to ensure submission of required documents and compliance with federal policies.

3. Develop, support and monitor progress of projects

a. Manage projects by having regular conference calls and in-person meetings

As noted above, project teams and the EC had monthly conference calls with progress being tracked and to-do-items clearly delineated by preparing and distributing minutes by the Associate Director. In addition, there is an in-person meeting every 2 weeks between all Cores at Southern Research, along with Investigators from Project 4.

b. Organize quarterly project reviews (face-to-face or teleconference) by the EC

At this point, the monthly update meetings serve the goal of informing the EC of progress and obtaining their input on decisions to be made.

c. Monitor overall Center research quality and progress annually by the EAB

The EAB met for the third time at the annual meeting in September 2016 and subsequently provided a report with advice at a high, project portfolio level. Two new additions to the EAB, Kara Carter, PhD, and Pei-Yong Shi, PhD, provided a fresh perspective on activities of AD3C.

d. Solicit additional regulatory guidance on an as-needed basis

As we are getting closer in certain projects towards animal models, we received regulatory guidance from the EAB to forego non-human primate studies; rather, show proof of concept in a rodent model, and then move into human studies as soon as possible.

e. Assist with identification and management of Intellectual Property developed by projects

As described in the consortium agreement, novel chemical scaffolds with potent antiviral activity will be protected, using Southern Research as the lead institution since they have the most expertise in this area. We expect to file at least one application in the next project period.

4. **Stimulate collaboration and synergy**
   a. Identify potential areas or topics of collaborations between projects and cores
      As mentioned earlier, a set of about 30 compounds has been identified to be attractive enough to be tested across all projects, to identify broad spectrum antivirals. For at least one of these compounds, the mechanism of action is being investigated by several project sites, to confirm and compare resulting potential target proteins.
   b. Provide and facilitate access to resources needed in the projects
      The Admin Core facilitated rebudgeting of available funds from Core A to Core B, since the screening library was expanded to cover more chemical space.
   c. Ensure that active hits in one project with potential against other viruses are evaluated in other projects
      As mentioned earlier, this is being actively pursued, under joint leadership with Core C
   d. Set up a data sharing and project tracking website
      We continue to use the Enterprise Content Management software "Documentum CenterStage". All the AD3C and EAB members have access to this secured site via a login name and password. Core C maintains a specialized database with more advanced tools to query structures, antiviral activity and many other parameters of the compounds.
5. **Facilitate meetings**
   a. Host monthly conference calls of project teams
      An audio and web meeting service available through AT&T has been used by Administrative Core personnel to host the monthly project team meetings.
   b. Organize conference call-based and in-person meetings of the project and core PIs (EC)
      In addition to getting together at the annual AD3C meeting in September, the EC met in April 2016, in conjunction with the International Conference for Antiviral Research in La Jolla, CA.
   c. Organize and implement Annual face to face meeting with all involved personnel, including the EAB
      The Admin Core has hosted AD3C's third annual scientific meeting, in Birmingham, AL, on September 15-16, 2016, to discuss the status of each of the projects. All our EAB members attended, as well as NIAID staff. Small group meetings were used to cover technical details of screening protocols and chemistry directions.
   d. Facilitate consulting and other scientific and professional meetings
      The projects are in an early phase of discovery and no consulting other than that received from the EAB has been required as of yet.
   e. Attend the annual CETR Program meeting and reverse site visit
      During this project year, NIAID staff joined our Annual Meeting, eliminating the need for a reverse site visit. With no CETR Program planned, ICAR served as a national meeting site.
6. **Manage Supplemental Research Projects applications**
   a. The Admin Core facilitated a request for proposals for supplemental research funding in the Spring of 2016; 7 applications were received from 5 different institutions, all of which were reviewed by at least 3 reviewers in the EC. The EAB received these reviews and ultimately selected 2 applications to be forwarded to NIAID for consideration; none were selected for funding.
   b. In addition, supplemental Zika funding was made available from NIAID; AD3C forwarded several proposals to NIAID for consideration, of which one was selected for funding: a screening campaign by SR, which is currently underway.
7. **Outreach to the public**
   a. Set up and maintain a website
      The Administrative Core maintains a website: www.uab.edu/ad3c, which contains descriptions of the Center, projects and cores and associated personnel, along with a publication list.
   b. Write press releases
      No press releases were required in this project period.
   c. Publish newsletters/ebriefs
      The Administrative Core has not had a need yet to publish an electronic "ebrief".
   d. Help with scientific publications
      The Admin Core continues to ensure acknowledgement of grant support and submission to PubMed Central and PMCID requirements.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Admin Core-8274

RESEARCH & RELATED BUDGET - SECTION A & B **FINAL**

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Richard	J	Whitley		PD/PI	(b)(4); (b)(6)				18,510.00	5,701.00	24,211.00
2.	Dr	Maaike		Everts		Associate Administrative Director					29,900.00	9,209.00	39,109.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **63,320.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	1 Program Manager, 1 Program Coordinator	(b)(4)			19,843.00	6,766.00	26,609.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**26,609.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**89,929.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	48,572.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**48,572.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	750.00
2. Publication Costs	0.00
3. Consultant Services	12,500.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Phone/Web Meeting Costs	3,155.00
9. Expenses for Annual Meeting	10,500.00
10. Website, Copying/Printing, Shipping	3,250.00
Total Other Direct Costs	**30,155.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**168,656.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	168,656.00	79,268.00
		Total Indirect Costs	**79,268.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**247,924.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Admin Core Budget Justification Yr 4 12-2016.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel

Richard J. Whitley, MD, PD/PI, (b)(4) months: Dr. Whitley will continue to serve as the Program Director/Principal Investigator of AD3C, and as Director of the Administrative Core. Dr. Whitley is currently Professor of Pediatrics, Microbiology, Medicine and Neurosurgery, and holds the Loeb Chair in Pediatrics in the School of Medicine at the University of Alabama at Birmingham (UAB). He will continue to manage and provide guidance and oversight to the AD3C investigators, communicate regularly with the NIAID Program Officer, members of the EC and Scientific Advisory Board, and work closely with the Associate Administrator and staff to ensure that planned project milestones are met. He will also continue to moderate monthly teleconferences, and lead Center annual meeting which was combined with the NIH reverse site visits for Year 3.

Maaike Everts, PhD - Associate Administrative Director (b)(4) months,
Dr. Everts is an Associate Professor in the Department of Pediatrics, School of Medicine at the University of Alabama at Birmingham. She continues to serve as the Associate Director of the Alabama Drug Discovery Alliance. She will continue to lead day to day research efforts of the AD3C, facilitating communication and interaction between project investigators and the cores, and serve as primary liaison between projects, providing research updates to the Director, Executive Committee and Scientific Advisory Board. She will also continue to work closely with the Program Director and Administrative Core staff to monitor the status of all projects and cores with respect to administrative, financial and regulatory aspects of the program.

Mary Wyatt Bowers, MA, (b)(4) months: Ms. Bowers will continue to oversee the financial administration of AD3C, and maintain responsibility for budgetary issues and invoicing, and coordinate with Sponsored Programs on subawards, and amendments. She will continue to aid Maaike Everts (see above) with meeting organization and planning, the annual noncompetitive renewal application and all other proposals related to AD3C funding. She also serves as the liaison with the UAB Office of Sponsored Programs, Grant Accounting, and Principal Investigators and institutions for matters related to financial and contractual agreements.

Sara Davis (b)(4) months, Ms. Davis is the program coordinator and administrative assistant to Dr. Rich Whitley. She will continue to assist Drs. Whitley and Everts with logistical aspects of meeting scheduling and organization as well as communications with external institutions and agencies and report preparations.

Consultants

Funds are budgeted for four members of the required external scientific advisory board to attend Center meetings and review activities of all projects. As projects enter year four, their continued guidance and oversight, and recommendations for the four projects remains of critical importance. The budget is based on estimated $2,500 reimbursement per advisor annually.

Supplies

Minimal funding is requested to provide for copier, postage, and office supplies needed to manage administrative activities of the large multi project program. Costs are estimated based on historical experience with similar multi-site project management.

Travel

Funds are requested for the following travel:

- Annual NIH reverse site visit expenses for the PD/PI, 1-2 project leaders and administrative staff in Rockville, MD.

- PD/PI, core personnel and/or Administrative Associate Director travel to provide technical assistance or oversight to one or more projects ($3,000)
- Executive committee meeting for PD/PIs and project leaders held approximately six months after Center Annual meeting.
- AD3C Center annual meeting held in Birmingham that includes PD/PIs, project leaders, postdocs, scientific advisory board members.
- Travel for project leaders and investigators to attend the annual International Conference on Antiviral Research (ICAR) to be held in LaJolla, CA on April 17-21, 2016. Based on discussions with the ICAR Program chair, who is also an AD3C investigator, a block of time has been set aside for the AD3C investigators to present their research. This meeting will replace the National CETR meeting which is no longer scheduled, per the November 2015 reverse site visit discussions.

Other Expenses

Teleconference/web meeting: Funds will continue to be used to cover the costs of monthly teleconferences and/or web meetings outlined in the administrative core plan to review and monitor projects and update project leaders on a continuous basis.

Computer Website design and maintenance: costs include continued website maintenance by a university professional to allow for communication and data sharing among investigators as well as providing a means for public access.

Publication/duplication costs: Funding is requested to cover costs related to publications and copying of draft progress reports, meeting minutes, and material used for annual or executive committee meetings. Costs are based on historical costs for meeting and reports used for a similar program.

Annual meeting expenses: funds are included to cover costs related to hosting a large Center meeting for investigators and scientific advisors. Based on suggestions following the recently completed annual meeting, it is expected that the meeting will be expanded to two full days in the coming year. The budget includes costs for meeting space, audiovisual services, incidental refreshments for attendees, and rental of items such as poster display boards.

A. COMPONENT COVER PAGE

Project Title: Project 1.1 Identification and Development of Anti-Flavivirus Lead Drug Candidates

Component Project Lead Information:

NELSON, JAY A

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Project 1 is designed to identify and characterize small molecule inhibitors of flaviviruses, a family of single stranded, positive sense RNA viruses that are associated with significant worldwide morbidity and mortality. This proposal builds on existing expertise in small molecule screening for DENV and is designed to identify small molecule compounds with the potential to be developed as antiviral agents. The initial screen in this proposal will focus on two medically relevant flaviviruses: dengue viruses (DENV) and West Nile virus (WNV). An existing screening platform will be adapted to screen multiple compound libraries, which include a high representation of nucleoside and nucleotide analogs, potentially compounds that have activity against multiple flaviviruses. If broad-spectrum leads with efficacy against multiple viruses can be identified, their further development will be emphasized. In order to enrich for potentially broadly acting compounds, we will focus on compounds that target one of the following important enzymatic activities of the flavivirus NS5 protein: the RNA-dependent RNA polymerase (RdRp), which is essential for replication of the viral RNA genome and the 2'-O-methyltransferase (2'-O-MTase), which is required for the virus to evade the host innate immune response. These activities are conserved among the flaviviruses, and similar activities are found in other virus families as well. The overall CETR proposal contains several projects focused on various virus families that are linked by a central screening facility and compound libraries. Therefore, the parallel screening strategies will maximize the likelihood of identifying broad-spectrum antiviral agents that may function across multiple virus families. The specific aims of Project 1 are:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds identify novel inhibitors of flavivirus replication.
Rationale: The Southern Research Screening Core (SR SC) has developed and validated cell-based, high-throughput assays for inhibitors of DENV and WNV induced cytotoxicity. Initial use of this, or similar, assays has already identified several compounds with antiviral activity. Therefore, this assay will be used to screen novel libraries that have not previously been extensively screened against human pathogens.
Experimental strategy: A CPE based assay will be used as a primary screen for compounds with anti-DENV or anti-WNV activity. Additionally, the WNV screen will be modified in order to allow the detection of compounds that inhibit the viral 2'-O-MTase, thereby sensitizing the virus to the actions of interferon and its effectors. Following the initial screen, "hits" will be evaluated in dose response and cytotoxicity assays in order to determine EC50, CC50, and selective indexes.
Aim 2: Characterize the antiviral activity of hit compounds
Rationale: Hit compounds will be further characterized with regard to efficacy and mechanism of action. The primary screen will potentially identify compounds that inhibit any of the stages of the viral replication cycle, therefore, secondary experiments are designed to elucidate the stage at which individual compounds act. Additionally, we will also characterize the compounds with regard to breadth of activity against other viruses, and examine the potential for evolution of compound-resistant mutants.
Experimental strategy: We will initially test compounds against sub-genomic viral replicons, which will identify compounds that do not function through affecting viral entry or egress, allowing us to focus on inhibitors of translation, protein processing, or RNA replication. We will also identify compounds that function through inhibition of the 2'O MTase, as well as compounds that act non-specifically through induction of interferon or other innate pathways. Compounds will also be evaluated in viral growth assays in order to evaluate the their effect on inhibition of production of infectious progeny virus. Additionally, we will analyze compound effects against multiple viruses and in multiple cell types. Finally, we will test the ability of the virus to develop resistance to individual compounds, as well as characterize any such mutants.
Aim 3: Chemical optimization and in vivo efficacy of lead compounds in animal models of West Nile and Dengue infection.
Rationale: Hit compounds identified and characterized above will be optimized to increase efficacy, selectivity, and bioavailability. These compounds will progress to testing in mouse models of infection.
Experimental strategy: Specific compounds and scaffolds will be triaged by the Medicinal Chemistry and Lead Development Core (MCLDC). Compounds with appropriate pharmacokinetic properties will be tested for prophylactic and therapeutic effects in mouse models of WNV and DENV infection.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 Goals Accomplished Whitley Nelson U19 RPPR Year 3.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B. 6 Plans for next year. Mechanism of action studies on selected active compounds will continue. In addition to sequencing of resistant mutants, we will employ a series of assays to determine the stage of the viral replication cycle that is targeted by the individual compounds. Time-of-addition studies will be used to determine when the drug must be present to effect inhibition. Results of these studies will suggest if the effect is primarily on viral entry or a later step. We will use of a sub-genomic replicon to examine the affect on viral protein expression and RNA replication. We will also assay production of infectious progeny by plasmid expression of the viral C, prM, and E proteins in cells expressing a reporter (GFP) replicon. The structural proteins are capable of packaging the replicon and are released as infectious particles that can transfer the replicon (and GFP+ phenotype) to naïve cells. This assay can be used to assess the effect of compounds on assembly and egress of viral particles as well as changes in the infectivity of progeny virus. We will additionally employ assays of viral attachment and entry if the time-of addition experiments suggest that the drug acts early in infection. These assays are currently established in our lab (PMID: 24599995).
We will also begin secondary screening of compounds identified in the WNV screen. As discussed above, IFIT1-dependent compounds will be the focus of the Diamond lab, while compounds that functioned +/- IFIT1 will be analyzed in the Hirsch/ Nelson labs. Compounds will be assessed for activity against WNV and DENV, as well as other flaviviruses, such as Zika virus. As always, compounds will be exchanged amongst labs within this project and other projects to identify potential pan-antiviral compounds, with SRI acting as the central distribution node.
Following completion of pharmaco-kinetic analysis of lead compounds, we expect that we will begin testing of lead compounds in animal models of infection. For analysis of DENV replication, these studies will be carried out in interferon-deficient AG129 mice.

B. 2 Accomplished under goals:

Major activities and objectives: The major activities for this reporting period have been: HTS for small molecule inhibitors of WNV in the presence or absence of the antiviral cellular protein IFIT1 conducted by SRI Core B, and continuing follow-up and characterization of compounds and compound analogs with anti-DENV and anti-WNV activity identified by SRI. We also continue to collaborate with project 3 (alphaviruses) to analyze compounds identified by SRI as alphavirus inhibitors for anti-flavivirus activity.

Results and outcomes:

WNV inhibitors: SRI Core B has completed the HTS screen for WNV inhibitors. This screen was carried out in matched cell lines in the presence or absence of IFIT1 (expressed via an inducible promoter). As detailed in the initial proposal, inhibitors that are dependent on the presence of IFIT1 are potentially acting against the viral 2'-O-methyltransferase (2'O-MTase). IFIT1-dependent compounds are undergoing secondary screens in the Diamond lab (Washington Univ), while inhibitors that act in the presence or absence of IFIT1 will be re-screened in the Nelson/ Hirsch labs (OHSU).

DENV inhibitors: Over the previous year, we have screened multiple lead compounds and analogs for activity against DENV. Secondary screens include immunofluorescent detection of the viral E protein in cultured cells and focus-forming assay to detect infectious progeny in culture supernatants. These screens are performed at 1 and 10 µM concentrations of each compound. Promising leads are then further evaluated in dose response assays in which concentrations range from 0.04 to 30 µM in order to calculated IC_{90} values. One such lead, SRI-33361 (Fig 1A) is a current focus of the SRI medicinal chemistry core. As shown (Fig 1B), SRI-33361 treatment of cultured cells results in a >100 fold decrease in viral titers at 10 µM and the IC value is ~0.5 µM. Several analogs of 33361 maintain similar activity, although others are inactive. Additional compounds shown include SRI-35847, and SRI-34963. Interestingly, SRI-34963 was identified by Project 3 (alphaviruses) as a potent inhibitor of chikungunya virus (CHIKV). This compound was re-screened for activity against DENV and found to be active. As with the alphaviruses, this is the most active analog within this series ("tetralins") of compounds.

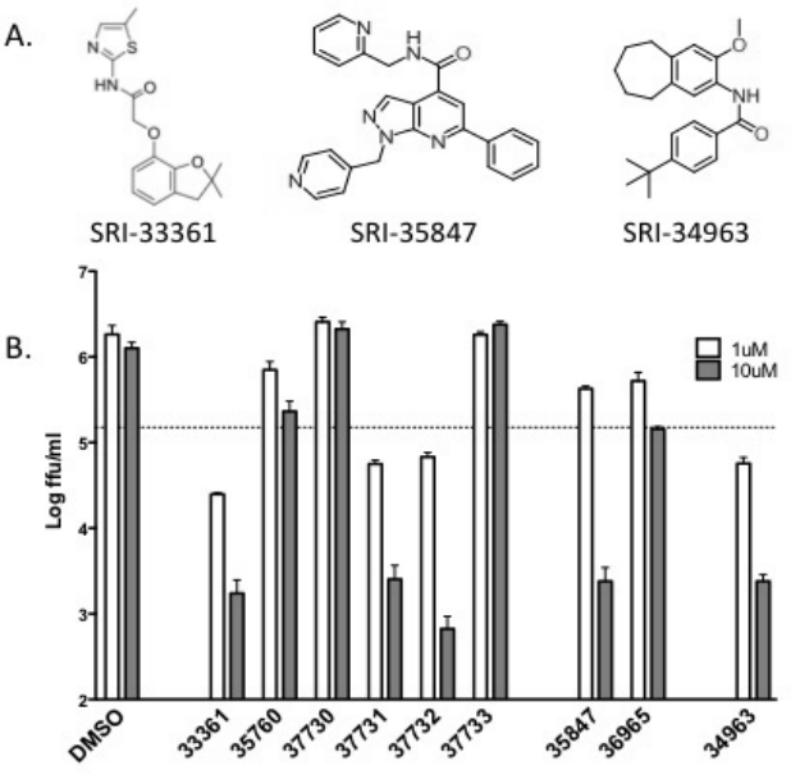


Fig 1. Inhibition of DENV-2. A. Lead compounds discuss text. **B.** HEK293 cells were infected with DENV2 (NGC) at a multiplicity of 0.1 ffu/cell. At 72 h pi, supernatant was harvested and viral titers measured by focus-forming assay on Vero cells. Lead compounds and respective analogs are grouped.

Screening will continue with new analogs in order to determine SAR, as well as to maximize compound efficacy and other desirable properties.

In order to begin to elucidate mechanism of action of individual compounds, we have passaged DENV continuously in the presence of individual inhibitors in order to elicit resistant mutants. Passage of DENV-2 in the presence of SRI-33361 for 7 rounds resulted in apparent drug resistance in 2 independent experiments (Fig 2). IC90 values for mutant viruses were calculated as ~14 µM, in contrast to 0.5 µM for the parental virus. We are currently generating clonal isolates of putative resistant viruses, which will be followed by sequencing. Nucleotide differences resulting in amino acid changes will be introduced into a DENV2 infectious clone. The clone will be used to generate viruses that will be analyzed for the resistant phenotype. If we are unable to recapitulate the resistant phenotype with single mutations, we will consider introducing multiple mutations, as well as analyzing nucleotide changes that appear in the 5' and 3' UTRs.

Challenges, solutions, and response to comments by external advisory board (EAB): A potential problem with lead compound SRI-35847 is a lack of IP space surrounding this molecule. Analog SRI-36965 (Fig 1) was created to avoid claimed IP space. In several experiments, this molecule has proved slightly less active than the parent compound. This may lead to a lack of emphasis on this compound series going forward.

Additionally, several suggestions were made by the EAB at the last AD3C meeting. One was the inclusion of a reference compound in dose response curves to serve as a positive control as well as to control for variability in the assay. We will therefore begin using compound NITD-008, an adenosine analog and inhibitor of DENV replication in these assays in addition to compounds of interest. Activity of NITD-008 in similar assays has been described (PMID: 19918064), so it will serve as an ideal reference compound. A second comment asked about our apparent reliance on resistant mutant analysis for mechanism of action studies. As detailed in the "plans for next year" section, we will employ a number of assays to elucidate the stage of the viral life cycle that is inhibited by active compounds. We generally begin resistant mutant studies first, due to the time taken to select for such viruses in culture.

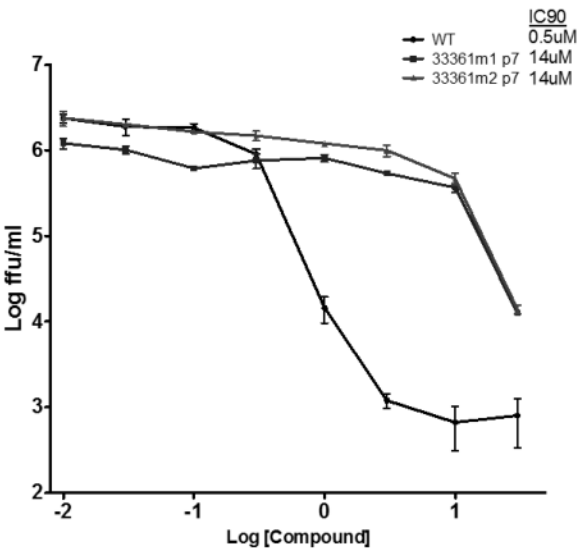


Fig 2. Passaging of DENV-2 in the presence of SRI-33361 results in resistant variants. Virus was collected after 7 passages in the presence of drug. Viral titer was determined and p7 virus or parental controls were grown on HEK293 cells (MOI=0.1) in the presence of indicated concentrations of SRI-33361. At 3 d pi, supernatants were collected and viral yield quantified by focus forming assay. IC_{90} values were calculated from resultant data.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Alec		Hirsch		Co-Investigator	(b)(4); (b)(6)						
2.	Dr	Jay		Nelson		Sub Award P.I.							
3.	Dr	Jessica		Smith		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **143,664.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical	(b)(4); (b)(6)					
3	Staff Scientist 3, Sr. Research Assoc., Lab Aide						
3	**Total Number Other Personnel**					**Total Other Personnel**	**66,730.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**210,394.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,500.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**6,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	31,545.00
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Charges, lease and per diem fees	19,600.00
9. Microscopy, sequencing	7,000.00
10. M-Chem Core Service	60,000.00
Total Other Direct Costs	**120,145.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**337,039.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	75.0	274,539.00	205,904.00
2. MTDC	26.0	62,500.00	16,250.00
		Total Indirect Costs	**222,154.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**559,193.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Whitley_Nelson_Project1_Budget_Justification_Year 4.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION, YEAR 4:

Nelson- Anti-flavivirus drug discovery

PERSONNEL:

Jay Nelson, Ph.D., Principal Investigator: (b)(4); (b)(6) Dr. Nelson is a senior molecular virologist with over 180 papers and reviews on a variety of topics, including herpesviruses, retroviruses, and flaviviruses. The primary focus of Dr. Nelson's research over the years has centered on the molecular pathogenesis and immune response to viruses including herpesviruses, flaviviruses and retroviruses. Dr. Nelson's group has used molecular and animal model approaches over the past 30 years to characterize cytomegalovirus (CMV) and flavivirus replication. Dr. Nelson's group, in collaboration with Klaus Früh and Alec Hirsch, used functional genomic approaches to determine that cYes, a cellular Src kinase, is an important regulator of flavivirus maturation. We have also shown that capsid interaction with cYes alters tight junction function by targeting degradation of Claudin 1 by the lysosome and have shown that WNV regulates the unfolded protein response (UPR) through CHOP to block cellular apoptosis. This project is the result of a long-term collaboration between Drs. Früh and Hirsch to identify potential lead compounds that inhibit Dengue and WNV replication. Dr. Nelson will be responsible for the planning of experiments and oversight on progress for this grant, as well as for communication with other Project and Core leaders and dissemination of results.

Alec Hirsch, Ph.D., Co-Investigator: (b)(4); (b)(6) Dr. Hirsch is an Assistant Scientist at the Vaccine and Gene Therapy Institute (VGTI) and will serve as Co-Investigator of the anti-flavivirus drug discovery project. Dr Hirsch has extensive experience with *in vitro* and *in vivo* models of flavivirus infection. He will be responsible for directing the investigation of the efficacy of compounds *in vitro* as well as *in vivo* in mouse models of viral infection. His duties will include coordination with other arms of this proposal, disseminating data sets produced during this project, and ensuring timely completion of the proposed work.

Jessica Smith, Ph.D., Co-Investigator: (b)(4); (b)(6) Dr. Smith received her Ph.D. in biomedical sciences from University of New Mexico School of Medicine in 2008, where she studied entry and trafficking of human papilloma virus. Since her time at the VGTI she has studied multiple aspects of flavivirus-host cell interactions, including identification and characterization of anti-flaviviral compounds. She will be responsible for directing lab personnel in conducting secondary and tertiary screens in conjunction with Dr. Hirsch, as well as supervising follow-up studies characterizing mechanisms of compound action. Additionally, she will be responsible for organizing and distributing data to other projects within the A3DC.

Meaghan Hancock, Ph.D., Staff Scientist 3 (b)(4); (b)(6). Dr. Hancock is a molecular virologist working with Dr. Nelson the VGTI. She will be responsible for conducting secondary and tertiary screens as well as conducting follow-up studies characterizing mechanisms of compound action. Dr. Hancock will also assist in the *in vivo* studies to be conducted in later years of this project.

Christopher Parkins, M.S., Senior Research Associate: (b)(4); (b)(6) Mr. Parkins will be responsible for producing WNV and DENV titered stocks for infection studies and assisting as needed with *in vitro* assays. Mr. Parkins will also be responsible for management of the AG129 mouse colony, infection of mice for *in vivo* studies, processing of animal samples, performing quantitative RT-PCR detection of virus in plasma and tissue samples from infected mice.

Erika Ferreira-Martine, Lab Aide: (b)(4); (b)(6) Ms. Ferreira-Martine will wash, organize and track all lab supplies for this project.

SUPPLIES:

Plasticware ($5,545/ yr Yr 1-5)

Disposable plasticware will be required for cell and virus culture, DENV and WNV titration and virus isolation, and molecular biological work. This includes tissue culture dishes of myriad sizes and layouts, flasks, serological pipettes, disposable pipette tips, microfuge and centrifuge tubes, and disposable screw cap tubes of various sizes for sample storage.

Tissue Culture Supplies ($6,000/ yr Yr 1-5)
These will be required for all cell growth and maintenance as well as virus growth and titration and isolation from tissues. This includes cell culture growth media, animal serum, PBS, trypsin, sucrose, sorbitol, disposable sterilizing filters, antibiotics, and syringes.

qRT-PCR (Taqman) ($10,000/ yr Yr 1-5)
qRT-PCR will be used for the detection of both WNV and DENV in animal tissues and quantitation of viral RNA replication in culture. Reagents for virus detection include: Reverse transcription reagents, ABI Master mix containing Taq polymerase, virus-specific primers and TaqMan probes, 96-well optical plates and caps.

Surgical Supplies: ($2,500/ yr Yr 1-5)
Vacutainer blood tubes, needles, syringes and sterile plastic collection tubes and swabs required for obtaining blood samples and tissues, isoflurane for anesthesia; Alzet osmotic pumps for cases in which continuous delivery of compounds is to be examined.

Enzymes/ molecular biology supplies/ chemicals: ($5,000/ yr Yr 1-5)
PCR reagents for cloning of viral mutants, restriction enzymes, Western blotting and protein analysis supplies, buffers, acrylamide, agarose.

Toxicity assays: ($2,500/ yr Yr 1-5)
Celltiter Glo reagent (Promega) for determination of toxicity of individual compounds.

Mice purchase ($6,000/ yr Yr 1-5)
We expect that we will require approximately 350 of each strain for the experiments described in this proposal. We will maintain a colony of AG129 mice to provide mice for DENV experiments. Calculation of number of breeding cages and cages to maintain weaned mice to support proposed experiments (according to “Breeding Strategies for Maintaining Colonies of Laboratory Mice.” Published 2007, The Jackson Laboratory) = 20 cages. We will purchase C57/Bl6 mice (3-4 weeks of age) from Jackson Laboratories ($16.40/animal) = approx. $6,000.

TRAVEL:
$6,500/year for Co-Investigators to attend an international meeting pertaining to antiviral therapeutics and vaccines directed against RNA virus infection and disease.

OTHER EXPENSES:
Mice per diem ($13,600/ yr Yr 1-5)
We expect that we will require approximately 350 of each strain for the experiments described in this proposal. We will maintain a colony of AG129 mice to provide mice for DENV experiments. Calculation of number of breeding cages and cages to maintain weaned mice to support proposed experiments (according to “Breeding Strategies for Maintaining Colonies of Laboratory Mice.” Published 2007, The Jackson Laboratory) = 20 cages. At $1/cage/day per diem = $7,300 annually. Cage costs for weaned mice and housing during experiments at $3.50/cage/day per diem (approx. 2 months per cage) = $4,200. We will purchase C57/Bl6 mice (3-4 weeks of age) from Jackson Laboratories. Per diem cage costs for these mice at $3.50/cage/day per diem should total $2,100 (assuming a total of 1 month housing).

Microscopy: ($2,000/ yr Yr 1-5);
Quantitative fluorescence microscopy will be used in secondary screens to evaluate compound efficacy. Immunofluorescent staining will be performed at VGTI and plates read by the automated fluorescence microscope at the Oregon Translational Research and Drug Development Institute (OTRADI). Cost is $50 for setup and $125/hour + 35% overhead.

Sequencing: ($5,000/ yr Yr 1-5)
At $500/sample, we will use deep sequencing to identify resistance mutations that arise due to compound treatment. We will sequence approximately 5 samples per year including both WNV and DENV isolates.

Publications ($2,000/ yr Yr 1-5)
For publication costs. We estimate 1-2 publications per year.

M-Chem Core Services ($60,000/ yr Yr 2-5)
M-Chem Core Services: ($60,000/yr, Years 2–5): Dr. Aaron Nilsen is Director of the OHSU Medicinal Chemistry Core facility (M-Chem Core). For this project, the M-Chem Core will design chemical biology experiments to help biological researchers investigate the mechanisms of action of small molecule antivirals. Additionally, the Core will synthesize analogs of small molecule antivirals for use in mechanism of action experiments. In terms of experience, the Core Director has more than 17 years of experience in chemical biology, medicinal chemistry, drug discovery and organic synthesis. Dr. Nilsen was the lead synthetic chemist on the multinational Medicines for Malaria Venture team that recently delivered a new quinolone-3-diarylether compound (ELQ-300) to the MMV for clinical development. Chemical reagents will be required to synthesize analogs including building blocks and solvents, which will be charged through the Core.

INDIRECT COSTS:
The bulk of the indirect costs ($205,904) are calculated at the rate of 75% (based on direct costs of $274,539) for work to be conducted at the OHSU West Campus. The remainder of the indirect costs ($16,250) are calculated at an F&A rate of 26%, which is the off-campus rate used for research projects at OHSU. It is OHSU's policy not to charge a significantly higher indirect cost rate on projects that transfer from outside entities, even though the work is done on-campus. Charging the full rate on this project would create a financial hardship in terms of achieving and completing the aims of the project. Therefore, a 26% modified total direct cost rate is used for that portion of the project ($62,500 direct costs: $60,000 for M-Chem core serves and $2,500 for travel) that will be conducted at the OHSU Medicinal Chemistry Core facility (transferred from the Portland VA Research Foundation, Inc., which is outside OHSU).

A. COMPONENT COVER PAGE

Project Title: Project 2.1 Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics

Component Project Lead Information:

(b)(6); (b)(3):7 U.S.C. § 3401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overall goal of Project 2 is to identify inhibitors of two highly conserved CoV processes, replication fidelity and RNA capping, that are essential for SARS-CoV virulence and survival in vivo. Multiple viral proteins and enzymatic activities are critical for these processes, including CoV 3'-to-5' exoribonuclease (fidelity; nsp14-ExoN) and 2'-O-methyltransferase (capping; nsp16-OMTase) activities. Consistent with the importance of these processes, we have shown that decreased replication fidelity and ablation of RNA capping through genetic inactivation of either ExoN or OMTase, respectively, results in replication competent viruses that are profoundly attenuated in vivo.

Aim 1. To identify and develop inhibitors of CoV high-fidelity replication. We will test the hypothesis that inhibitors of CoV high-fidelity replication will decrease viral fitness alone and in combination with RNA mutagens, and represent potent pan-CoV therapeutics. In part 1, we will identify ribonucleoside analogs that inhibit CoV replication, and define their mechanism of action. High-throughput screening in part 2 will identify small-molecule inhibitors of CoV fidelity. In part 3 we will identify the viral protein targets of lead compounds, and determine their mechanism of fidelity impairment. In part 4, will we test highly efficacious compounds identified in parts 1 and 2 across the CoV family and viral platforms within this program.

Aim 2. To identify and develop inhibitors of CoV RNA capping activity. We hypothesize that small molecule inhibitors of essential CoV RNA capping components will profoundly increase CoV sensitivity to the host innate immune response through interferon-stimulated effectors. In part 1 we will use targeted mutagenesis of known CoV capping components to define distinct mechanisms to increase CoV sensitivity to the host ISGs. In part 2 we will examine the combined efficacy of known O-MTase inhibitors and type I IFN treatment against SARS-CoV, and perform a high-throughput screen for inhibitors of CoV RNA capping. In part 3 we will identify the viral protein targets and mechanism of action of lead compounds. In part 4, lead compounds will be tested across the CoV family and specific viral platforms within this program.

Aim 3. To chemically optimize and test the in vivo efficacy of CoV fidelity and RNA capping inhibitors. We will test the hypothesis that inhibitors of CoV fidelity or RNA capping are highly attenuating in vivo and represent broadly effective CoV therapeutics. Compounds identified in Aims 1 and 2 will be chemically optimized for in vitro efficacy, selectivity, solubility, microsomal stability, and bioavailability at SR. Using these optimized compounds, in part 1 we will confirm the biological target(s) of lead fidelity and RNA capping inhibitors in vivo. In part 2 we will test the efficacy of lead compounds against mouse-adapted SARS-CoV in progressively stringent mouse models of acute and persistent human disease. Efficacy will be determined by monitoring respiratory function, morbidity and mortality, histology, and viral replication. In part 3 we will test for the development of drug resistance in vivo, and will determine the efficacy of lead compounds against MERS-CoV and other CoV family members.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Project 2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4 Vanderbilt.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans for the next reporting period are based on continuation of the sections above. Briefly, hits from the SRI HTS will be assessed in vitro with MHV followed by SARS and MERS and if promising, compounds will be evaluating for efficacy within in vivo models of CoV pathogenesis. The continued preclinical development of GS-5734 will be the main focus of both (b)(6); and Baric labs. Given that the resistance mutations generated thus far only provide a moderate shift in EC50 (5-fold), the (b)(6); (b)(3):7 U.S.C. § 8401 will continue to select for mutations that further enhance resistance for both SARS- and MERS-CoV. In addition, the will lead efforts to determine the mechanism of action for GS-5734. The Baric lab will continue to assess GS-5734 efficacy in primary human cells that drive SARS- and MERS-CoV pathogenesis. In additional focus will be in vivo efficacy studies with genetically divergent bat CoV and MERS-CoV in newly developed transgenic mouse models. Our work on GS-5734 thus far will serve as key data for the preclinical package to be submitted by Gilead for IND licensure in the last quarter of 2016.

1.Preclinical development of GS-5734 in partnership with Gilead (b)(6); (b)(3):7 U.S.C. § 8401 nd Baric Labs.

(b)(6); (b)(3):7 U.S.C. § 8401

•Force MHV adaptation to increasing concentrations of GS-5734 or its parent compound to identify the maximum achievable level of resistance and associated additional mutations in the RdRp or other replicase proteins, thereby further revealing GS-5734 mechanism of action and CoV pathways to resistance.
•Select, genotypically analyze, and phenotypically characterize GS-5734-resistant MERS-CoV and SARS-CoV mutant viruses in Calu-3 cells. Evaluate impact of GS-5734 resistance mutations on replicative fitness of MHV, SARS-CoV, and MERS-CoV in cell-culture.
•Systematically examine steps and processes in the CoV life cycle affected by GS-5734 using wild-type, GS-5734-resistant, and RNA proofreading-impaired viruses.
•Select, genotypically analyze, and phenotypically characterize EIDD-1931-resistant MHV, MERS-CoV, and SARS-CoV mutant viruses. Determine the nature of functional interactions—synergistic, additive, indifferent, or antagonistic—between GS-5734 and EIDD-1931 when combined against wild-type, GS-5734-resistant, or EIDD-1931-resistant MHV, MERS-CoV, and SARS-CoV.
•Determine sensitivity of GS-5734-resistant mutant viruses to other classes of antiviral compounds, including mutagens (e.g., 5-fluorouracil, ribavirin, and 5-azacytidine) and inhibitors of RNA polymerization (e.g., 2' C-methyl adenosine).

Baric Lab

•Evaluate antiviral efficacy of SARS- and MERS-CoV in various primary human cells that guide in vivo pathogenesis. Antiviral assays in primary human type II pneumocytes, lung fibroblasts and endothelial cells, etc.
•Perform EC50/EC90 studies in HAE with parent/prodrug GS compounds that were effective at 1uM or less in previous pilot studies.
•In vivo efficacy studies with MERS-CoV in newly generated transgenic mice (Ces1c-/-/288/330+/+).
•In vivo efficacy studies with SARS-like bat CoV (HKU3) and MERS-like bat CoV (HKU5).
•Evaluation of in vivo pathogenesis of SARS- and MERS-CoV resistance mutants and assess their ability to counteract treatment with GS-5734 in mice.

2.Evaluation of hits from SRI HTS in partnership with SRI, UAB, (b)(6); (b)(3):7 and Baric Labs.

•Confirmation of antiviral activity of hits from HTS with MHV, SARS- and MERS-CoV (b)(6); (b)(3):7 U.S.C. § 8401
•Build SAR for additional medicinal chemistry efforts on current leads (SRI (b)(6); (b)(3):7 U.S.C. § 8401
•For lead candidates, initiate passage for resistance, deep sequencing and possible mechanism (b)(6); (b)(3):7 U.S.C. § 8401
•Testing in HAE cells of verified active compounds (Baric).
•Establish possible candidates for in vivo (mouse model) testing (Baric)

Project 2: Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics (b)(6); (b)(3):7 U.S.C. § 8401 Ralph S. Baric)

B.2. What was accomplished under these goals?

B.2.a. Major activities, specific objectives, significant results and key outcomes. Our collaborations with Southern Research Institute (SRI) and Gilead Sciences (GS) continue to generate significant and exciting results. Our major activities within the past reporting period include: 1) completion of a high-throughput screen (HTS) at SRI identifying two lead compounds 2) determination that Gilead compound GS-5734 is broad-spectrum with family-wide activity against CoV in human primary airway epithelial (HAE) cell cultures 3) completion of extensive metabolic and pharmacokinetic profiling of GS-5734 in collaboration with GS and demonstration that GS-5734 administered prophylactically or therapeutically improves lung function, lowers virus lung titers, and protects against weight loss and 4) determination that transfer of resistances mutations identified in mouse hepatitis virus (MHV), V553L and F476L, to SARS-CoV transfers the resistance phenotype and that these resistance mutations do not attenuate SARS-CoV pathogenesis in mice and 5) preparation of related grants and manuscripts.

1. SRI HTS reveals hits against SARS-CoV. The main objective of our collaboration with SRI was to perform an unbiased high-throughput screen (HTS) to identify compounds with antiviral activity against SARS-CoV. The initial CPE based HTS of >200K compounds identified 6 compounds with robust antiviral activity that were confirmed with completely separate batches of compound. Medicinal chemistry was performed at SRI to optimize the chemical properties (solubility, microsomal stability, etc.) while retaining antiviral activity. These efforts yielded two compounds, 33911 and 36565, with acceptable EC_{50} values while only one had decent solubility (36565). In the coming year, the antiviral activity of 36565 will first be confirmed and validated in vitro with model CoV (mouse hepatitis virus, MHV) and then evaluated with both SARS- and MERS-CoV in a human lung epithelial cell line (i.e. Calu-3 2B4) and if successful in primary human airway epithelial cells (HAE). If proven to be robustly antiviral against SARS- and MERS-CoV in vitro, SRI molecules will be evaluated in vivo in mouse models of CoV pathogenesis.

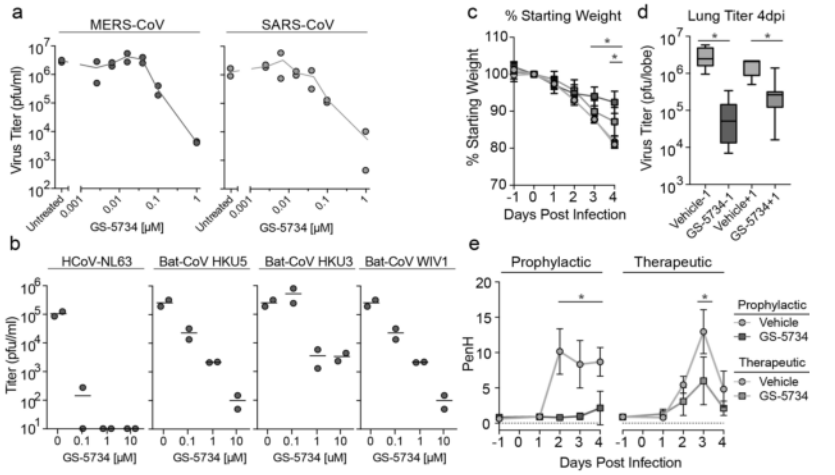


Figure 1: Antiviral efficacy of GS-5734 in primary human airway epithelial (HAE) cell cultures and mice. A) MERS- and SARS-CoV-infected HAE (MOI = 0.5) treated with increasing doses of GS-5734. B) HAE treated and infected as in Panel A. Group 1 human CoV NL63, group 2C MERS-like bat CoV HKU5, divergent group 2b bat CoV HKU3, and SARS-like prepandemic bat CoVs WIV1. Vehicle or GS-5734 (25 mg/kg) was administered twice daily beginning either day -1 or day +1 post-infection. C) Percent of starting weight demonstrating protection from weight loss with GS-5734 treatment. D) SARS-CoV titer in the lung is reduced with GS-5734 treatment. E) Pulmonary function as measured by whole-body plethysmography. Penh is a measure of airway obstruction.

2. GS-5734 is a broad-spectrum antiviral against CoV (*Baric Lab* (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 The main objective of our collaboration with Gilead has been to accelerate the preclinical evaluation of antiviral compounds against CoV. We have evaluated almost 30 different parent or prodrug nucleoside analogs for antiviral activity against SARS- and MERS-CoV in HAE with the parent GS-441524 and prodrug GS-5734 being the most promising. GS-5734, currently in clinical development (Phase II trials) for treatment of Ebola virus disease, inhibits SARS-CoV and MERS-CoV replication in multiple in vitro systems including HAEs with submicromolar EC_{50} values (SARS- and MERS-CoV EC_{50} = 30nm, **Fig. 1a**). GS-5734 was also effective against bat-CoVs, prepandemic bat-CoVs and circulating contemporary human CoV in primary human lung epithelial cells, thus demonstrating broad-spectrum anti-CoV activity (**Fig. 1b**). These data demonstrate that the prodrug, GS-5734, is efficiently transported and metabolized by key target cells of both SARS- and MERS-CoV in vivo. To ensure that phenotypes observed in primary human cells were not donor-specific, compounds are evaluated in cells from at least three donors. Since both SARS- and MERS-CoV target multiple cell types in the lung, future efforts will focus on determining antiviral efficacy in key primary cell types that mediate in vivo pathogenesis (i.e. type II pneumocytes, lung fibroblasts and endothelial cells, etc.).

3. Both prophylactic and therapeutic GS-5734 protects mice from SARS-CoV induced disease *(Gilead and Baric Lab, Sheahan).* To accelerate the preclinical development GS-5734, in collaboration with GS, we have performed extensive in vitro metabolism and in vivo pharmacokinetic (PK) analysis. GS-5734 has relatively poor plasma stability in mice due to expression of a secreted carboxylesterase 1c (*Ces1c*) absent in humans and non-human primates. Thus, all PK and antiviral efficacy studies were performed in *Ces1c*$^{-/-}$ mice. These studies demonstrated that drug was metabolized to the active triphosphate (TP) in the lungs of mice administered GS-5734 subcutaneously and that a twice daily dosing (BID) regimen was able to maintain trough levels consistent with those anticipated in humans and sufficient to maintain CoV inhibition over the dosing interval (data not shown). Importantly, with both prophylactic (i.e. dosing beginning 1 day before infection) and therapeutic (i.e. 1 day post infection) BID dosing via the subcutaneous route, we demonstrated antiviral efficacy against SARS-CoV with significant reductions in titer, improved lung function and minimal weight loss with treatment as compared to vehicle treated controls (**Fig. 1c-d**). The observed antiviral effect was independent of sex and age. Both SARS- and MERS-CoV pathogenesis is increased with increasing age in humans and this phenotype can be modeled in adult and aged mice. Thus, protection of both adult (20-28 week old, Fig. 1c-d) and aged (60+ week old, data not shown) suggests that GS-5734 treatment in even the most vulnerable human populations may be effective. Since the murine ortholog of the human MERS-CoV receptor (DPP4) does not support MERS-CoV infection, in collaboration with Gilead, we are generating a mouse deficient in *Ces1c*$^{-/-}$ containing human DPP4 alleles at positions 288 and 330 (*Ces1c*$^{-/-}$/288/330$^{+/+}$) to facilitate in vivo efficacy studies with MERS-CoV. In addition, we have additional mouse models for genetically divergent bat CoV (HKU3 and HKU5) within which we will assess the breadth of efficacy in vivo.

4. Resistance phenotypes are genetically transferable among CoV (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 *Baric Lab*, (b)(6); (b)(3):7 *Sheahan)* MHV serves as a highly informative, tractable, and efficient BSL-2 model system for studies of CoV genetics, replication mechanisms, and viral evolution. The (b)(6); (b)(3):7 U.S.C. § 8401 has shown that MHV can be used to rapidly screen compounds for potentially broad activity against divergent CoV's. Through selection and genotypic and phenotypic analysis of MHV isolates resistant to antiviral compounds, the (b)(6); (b)(3):7 U.S.C. § 8401 has obtained insights into mechanisms of action and genetic barriers to resistance. Of crucial importance, mutations in the MHV RdRp that confer resistance to GS-5734—V553L and F476L—recapitulate the resistance phenotype when introduced into SARS-CoV. As amino acid residues at these positions are absolutely conserved across diverse CoV's, we fully expect that MERS-CoV and other human and bat CoV's engineered with the V553L and F476L substitutions will phenocopy MHV resistance to GS-5734. We further anticipate that MHV will usefully serve to screen other lead compounds for anti-CoV activity, elucidate viral targets of these agents, identify potential pathways leading to resistance, and illuminate means of preventing or therapeutically circumventing selection of drug-resistant variants. To the latter point, the (b)(6); (b)(3):7 U.S.C. § 8401 recently made the exciting observation that V553L and F476L mutations, while fostering resistance to GS-5734, actually enhance MHV susceptibility to a cytidine analog developed at the Emory Institute for Drug Discovery, EIDD-1931. This complementary activity relationship between GS-5734 and EIDD-1931 demonstrates the potential to treat CoV infections with drug combinations that prevent emergence of clinical resistance. The (b)(6); (b)(3):7 U.S.C. § 8401 will continue using MHV to initially evaluate the impact of resistance mutations on the efficacy and potency of structurally similar and dissimilar compounds with anti-CoV activity, followed by testing of promising drug combinations against human CoVs.

5. Preparation of related grants and manuscripts. To accelerate the preclinical development of GS-5734 for the MERS-CoV indication and IND licensure, we have applied for a Partnerships for Countermeasures Against Select Pathogens (R01, RFA-AI-16-034) grant. We have also submitted a publication detailing our in vitro and in vivo evaluation of GS-5734 titled "Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses" to Science Translational Medicine. An additional manuscript describing resistance to GS-5734 is in preparation.

B.4. Opportunities for Training. Graduate students are active in the project at Vanderbilt. Individual development plans (IDPs) are generated on an annual basis for all graduate students. They are used for defining key objectives and goals for progress and for review on at least an annual basis. For the AD3C program, IDPs include specific goals relevant to the project. These assist in analysis of progress in projects and future training and career development. Construction of the IDP includes creation, review, and updating of biosketches and CVs; these serve as learning tools for presenting professional training and accomplishments in formats relevant to research funding proposals.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0044134560000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	(b)(6); (b)(3):7 U.S.C. § 8401				PD/PI	(b)(4); (b)(6)				46,275.00	5,506.73	51,781.73

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **51,781.73**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	(b)(4)					
1	Graduate Students				7,272.00	0.00	7,272.00
	Undergraduate Students						
	Secretarial/Clerical						
3	Research Asst., Sr. Resrch. Specialist, Sci./Lab Mgr.				114,761.58	26,643.96	141,405.54
4	**Total Number Other Personnel**					**Total Other Personnel**	**148,677.54**
						Total Salary, Wages and Fringe Benefits (A+B)	**200,459.27**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0044134560000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0044134560000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	40,632.73
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Repairs and Maintenance	10,000.00
Total Other Direct Costs	**52,632.73**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**256,092.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	58.0	256,092.00	148,533.00
		Total Indirect Costs	**148,533.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Steven Zuraf, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**404,625.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget Justification(b)(6); (b)(3):7 U.S.C. §.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION ((b)(6); (b)(3):7 U.S.C. § 8401 **AB, PROJECT YEAR 4)**

PERSONNEL

(b)(6); (b)(3):7 U.S.C. § 8401**, Principal Investigator** (b)(4) **months)** (b)(6); (b)(3):7 U.S.C. § 8401 will continue to serve as the Principal Investigator of Project 2. (b)(6); (b)(3):7 U.S.C. § 8401 studying coronavirus replication and replicase nonstructural protein functions. (b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401 **(Research Associate Professor,** (b)(4) **months).** Dr. (b)(6); (b)(3):7 U.S.C. § 8401 He has over 20 years of virology experience. (b)(6); (b)(3):7 U.S.C. § 8401 Vanderbilt BSL3 - SARS-CoV and MERS-CoV research program, (b)(6); (b)(3):7 U.S.C. § 8401 s an authorized Select Agent user, and will coordinate all investigators and studies in the BSL3 and will directly perform portions of studies in Aim 1, including additional testing of candidates from SRI and Gilead (b)(6); (b)(3):7 will direct all studies of engineered SARS-CoV and MERS-CoV. Additionally, (b)(6); (b)(3):7 U.S.C. will interact with collaborators for data analysis, presentation and publication.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) **months).** (b)(6); (b)(3):7 U.S.C. § 8401 s currently a (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 who has published experience working with emergent viruses. (b)(6); (b)(3):7 will carry out experiments from Aim 1 that introduce resistance mutations in the nsp12 RdRp. (b)(6); (b)(3):7 is an authorized Select Agent user.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) **months)** (b)(6); (b)(3):7 U.S.C. § 8401 s a (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 who has worked with (b)(6); (b)(3):7 U.S.C. § 8401 has extensive experience in performing mutagenesis, and in engineering recombinant viruses using our reverse genetics system. (b)(6); (b)(3):7 will carry out support experiments at BSL2 with mutagenesis, preparation of fragments, sequencing of MERS-CoV RNA and analysis of data. (b)(6); (b)(3):7 will coordinate all purchases

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) **months):** (b)(6); (b)(3):7 U.S.C. § 8401 is a research assistant 1 who has developed and optimized the luciferase containing MHV for initial testing of candidate inhibitors from Gilead Sciences and SRI. (b)(6); (b)(3):7 will continue to perform initial testing and generation of virological growth curves and EC50 analysis of initial and optimized inhibitors. (b)(6); (b)(3):7 also is responsible for cell culture preparation for all studies that will be performed at BSL3. (b)(6); (b)(3):7 also performs initial mutagenesis of luciferase containing genome fragments for MHV, SARS-CoV and MERS-CoV for use in generating recombinant viruses. (b)(6); (b)(3):7 will perform experiments, generate data, and analyze data in consultation with Dr. (b)(6); (b)(3):7 U.S.C. § 8401 related to passaging for resistance.

FRINGE BENEFITS: Fringe benefit calculations are derived from the current Vanderbilt University Medical Center guidelines.

LAB SUPPLIES ($40,633)

Cell Culture Supplies, Serum and Media ($10,000): A large amount of cell culture work is associated with the project, requiring media, serum, and culturing flasks. Consequently, funds are requested for media, serum, and related cell culture supplies to maintain Vero cells in culture, measure sensitivity to nucleoside analogs, and confirm target compounds. Reagents for the extended passage of SARS-CoV in the presence of nucleoside analogs and lead compounds to test for the development of resistance from Aim 1 will be required.

BSL3 Supplies, protective gear, disinfectants, ($20,745). All testing of nucleoside analogs, monitoring the development of drug resistance, and confirmation of lead compounds for SARS-CoV and MERS-CoV will be performed under strict BSL3 protocols. This will include extensive use of plastic ware, tissue culture reagents,

materials for plaque assays, and RNA isolation. BSL3 PPE (personal protective equipment) is also required for all work done at BSL3, Regular delivery of CO_2 for the incubators is also needed. In addition, supplies for analysis of RNA and protein at BSL2 as well as materials for shipping of samples between UNC and Vanderbilt are required. Separate reagents are needed for analysis of SARS-CoV RNA at BSL2 because the RNA is a Select Agent.

Enzymes and Reagents ($9,888): Any potential resistant mutations will need to be reengineered into the SARS-CoV reverse genetics clone. Generating mutations within the plasmids carrying fragments of the viral genomes will require the enzymes and reagents necessary for these molecular biology protocols. Assembling recombinant SARS-CoV requires large amounts of highly expensive restriction enzymes (e.g., BsmBI, etc.) and large amounts of DNA ligase. DNA markers are needed for identifying appropriately sized bands and assembly intermediates and full-length DNA products in gels; a critical step during the assembly of full-length cDNA clones. In addition, high quality T7 RNA polymerase is needed for driving production of full-length RNA transcripts for electroporation into susceptible cells and for the subsequent recovery of recombinant viruses. Cytotoxicity kits (e.g., CellTiter-Glo) as well as the 96-well plates and other plastic ware will be used to examine the toxicity of compounds identified in Aim 1.

REPAIRS AND MAINTENANCE ($10,000): This includes as is an annual decontamination and complete recertification of the BSL3 lab. This recertification includes required maintenance on any equipment within the BSL3. In addition replacement of at least 4 HEPA filters is required on a yearly basis. Service contracts for major equipment (autoclaves, incubators, centrifuges) are also required.

PUBLICATIONS ($2,000): Sufficient for two 2 publications per year from Aims 1 and 3

TRAVEL ($3,000). The budgeted amount will allow travel for participating investigators to UAB for the annual CETR meeting, and for one or two investigators to present at the American Society for Virology or the International Congress on Antiviral Research.

A. COMPONENT COVER PAGE

Project Title: Project 3.1 Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses

Component Project Lead Information:

STREBLOW, DANIEL N

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphaviruses are broadly comprised of geographically derived clades: New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinct pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean and Caribbean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America. Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease. Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication, demonstrating that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease. Since the target of this class of inhibitors, namely RNA- dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds that impact these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 3), Coronaviruses (Project 2), and Influenza (Project 4). In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating in vitro replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV. Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique molecular libraries. Our goal is the identification of lead molecules for further in vivo evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents. In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.
Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity. Initial use of this assay has already identified several compounds with antiviral activity against VEEV. Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.
Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the Alphaviruses VEEV and CHIKV. Following these initial screens, “hits” will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC50 , CC50 , and selective indices.

Aim 2: Validate and characterize antiviral activity and off-target effects.
Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to efficacy and mechanism of action.
Strategy: We will use a variety of secondary assays to identify: 1) breadth of anti-Alphavirus activity (test multiple Alphavirus species); 2) cell type-specificity (biologically relevant cells); 3) targets of antiviral compounds; and 4) ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of in vivo efficacy of lead compounds.
Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus. Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.
Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 Project 3.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans For Next Year:
1.Quinolones (SR-33394): Identify the mechanism of action for this class of compounds using in vitro assays. These will be tested for activity. Colorado will characterize VEEVTC83 virus that displays resistance to SR-34329 by utilizing sequence information generated by UNC to introduce the mutations into the infectious clone for VEEVTC83. SR plans to synthesize a new series based upon current SAR. Further SAR and testing will be dependent upon the results of the new analog series.
2.Tetralins-Benzo Annulenes (SR-33366): Additional SAR for this chemical series will be completed in order to optimize activity with stability and bioavailability. We plan to finish mechanism of action (MOA) studies for this compound series utilizing resistance phenotype information, in vitro assays and protein/compound binding. Since this compound series displays activity against a wide range of virus families; we will further characterize this property in order to determine the range of activity. In vivo pharmakokinetic (PK) analysis will be completed allowing us to determine dosing amount, route and timing for efficacy studies in our mouse models of CHIKV infection and disease.
3.VEEV 2015 HTS: SRI-36426 and SR-36427 were chosen for SAR due to their high activity and low toxicity profiles. SR will continue to provide the group with additional analog for these two scaffolds for SAR. PK analysis will be preformed on the most active compounds. Since SR-36426 is broadly active against 5 different Alphaviruses; we will determine the breadth of activity against other viruses. MOA studies should be completed for SRI-36426 and SR-36427.
4.CHIKV 2015 HTS: SR-36767 & SR36768 are the new leads for CHIKV but both compounds block a number of other Alphaviruses. We will continue to perform MOA and breadth of action studies for these two series. SR will continue to synthesize new analogs in order to optimize compound activity and stability. We hope to perform PK analysis and in vivo testing for these two series.
5.Project 1, 2, 4 Hits: We will continue to test additional compounds that are active against viruses from the other projects in order to identify broadly active compounds. We plan to determine the MOA for DENV compound SR-37014 because this was the most active compound identified in the cross-screens performed in 2016.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

Progress towards our goals is outlined for each Specific Aim:

SA1. HTS Screen of Novel Drug Libraries for Antiviral Compounds that Block Alphavirus Replication

1. 2015 Primary Screen: *VEEV HTS* identified 940 active samples and 8 out of 12 sent to OHSU had activity in NHDFs. *CHIKV HTS* identified 2,558 active compounds and 5 out of 11 were confirmed.
2. SR screened 347,000 compounds against $VEEV_{TC83}$ using Vero cells and 105 hits were identified. OHSU tested 35 and found 4 actives against CHIKV. SR derived analogs of two compounds (Tetralin-SR-33366 and Quinolone-SR-33394), which have been used for SAR and mode of action studies.
3. In order to both exclude compounds that block virus replication via activation of type I IFN responses and to enhance virus replication, Dr. DeFilippis constructed telomerized human foreskin fibroblast cells that lack IRF3 (THF-ΔIRF3). OHSU validated four anti-VEEV compounds as effective against CHIKV in these cells.
4. Construction and Sequencing of New CHIKV and VEEV Strains: The Alphavirus group has constructed new strains that will facilitate HTS and SAR including a new CHIKV strain expressing nano-Luciferase provided by UNC. Other recent isolates from Puerto Rico have been cloned and sequenced. These highly relevant strains may be used in subsequent validation experiments.
5. $VEEV_{TC83}$ has also been modified to encode nluc and is currently being validated at Colorado. $VEEV_{TC83}$-nLuc will be used by SR for SAR studies and the group for antiviral validation studies.

SA2. Validate and characterize antiviral activity and off-target effects

1. The group has developed multiple assays for secondary validation screens and to identify the mode of action for the lead hits. To prevent duplication of effort and maximize experimental efficiency, each individual laboratory of the Alphavirus group has undertaken the optimization of specific assays that they will utilize to test lead compounds.
2. **Quinolones (SR-33394):** SR synthesized 89 analogs. OHSU tested them in virus reduction assays and found the active compounds SR-33394 (EC_{90}=0.77μM), SR-34329 (EC_{90}=0.12μM), SRI-36506 (EC_{90}=4.9μM) and SR-36959 (EC_{90}=0.78μM). All other analogs had decreased activity compared to SR-33394. Colorado generated a $VEEV_{TC83}$ virus that displays resistance to SR-34329, which is being sequenced by UNC.
3. **Tetralins-Benzo Annulenes (SR-33366):** SR synthesized >73 analogs of SR-33366 for SAR. SR-34963 was found to have about a 10-fold increase in activity against CHIKV with an EC_{90}=0.45μM compared with SR-33366 (EC_{90}=3.2μM). Sequencing of UNC- and OHSU-derived resistance mutants identified changes in the macrodomain of NSP3. This finding is consistent with mode of action studies showing that SR-34963 blocks viral RNA synthesis at the level of subgenomic RNA synthesis. SR is performing crystallization/binding assays with NSP3. Analog SR-36429 (EC_{90}=1.5μM) showed better microsomal stability and PK activity and may be used for *in vivo* activity experiments. Additional recent analogs show activity and are under SAR. SR-34963 is also active against Flaviviruses (DENV, ZIKV), Coronaviruses, and Influenza virus.
4. **VEEV 2015 HTS:** OHSU confirmed 8 of 12 active hits including: SR-36415 (IC_{90}=0.77μM), SR-36416 (IC_{90}=0.35μM), SR-36420 (IC_{90}=0.13μM), SR-36421 (IC_{90}=0.11μM), SR-36423 (IC_{90}=0.22μM), SR-36424 (IC_{90}=0.06μM), SR-36426 (IC_{90}=0.72μM), and SR-36427 (IC_{90}=0.25μM). SRI-36426 and 27 were chosen for further SAR. SR-36426 is active against 5 different Alphaviruses and blocks infection prior to viral RNA synthesis. SR-36427 is active against VEEV and Mayaro virus and blocks infection after RNA synthesis. Both work in $IRF3^{-/-}$ fibroblasts indicating that they do not function through IFN.
5. **CHIKV 2015 HTS:** OHSU confirmed 5 of 11 including: SR-33001 (IC_{90}=0.93μM), SR-35756 (IC_{90}=3.39μM), SR-35894 (IC_{90}=0.75μM), SR-36767 (IC_{90}=0.09μM), and SR-36768 (IC_{90}=0.23μM). Two compounds (SRI-33001 and -36768) were active against 5 different Alphaviruses and SRI-36767 was active against 4 Alphaviruses. SR-36767 & -68 are the new leads for CHIKV and block infection prior to RNA synthesis. SR-33001 blocks viral replication at a step after viral RNA synthesis.
6. **Project 1, 2, 4 Hits:** DENV compound SR-37014 (IC_{90}=0.4μM) was active against CHIKV. SARS compounds SR-35742, -35894 and -36565 showed activity against VEEV but not CHIKV.

SA3. Chemical optimization and determination of *in vivo* efficacy of lead compounds

The group has developed a number of models to test *in vivo* efficacy of lead compounds. These include models of: 1) Acute CHIKV infection and joint disease, including models of disseminated inflammation based on unique mouse strains from the Collaborative Cross Mouse Genetics Resource as well as the generation of a mouse-adapted CHIKV strain developed at Colorado that displays enhanced replication, dissemination, and pathogenicity in WT mice; 2) Intranasal inoculation of VEEV for neurological infections; 3) chronic CHIKV infection and joint disease in wild-type and immunodeficient mice; 4) Lethal CHIKV and VEEV mouse models; and 5) CHIKV infection of nonhuman primates. Each lab has unique models that they will use for testing lead compounds; this will increase our ability to quickly determine the *in vivo* efficacy profile for each lead.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Research Material	1.THF-IRF-3: Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These cells constitutively express the reverse Tet-transactivator via lentivector (Clontech # 631069). The IRF3 gene sequence has been disrupted using the CRISPR/Cas9 system (AddGene vector # 49535). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 106 cells per vial. Cryopreserved cells can be brought up directly into a T75 + 14 mL media. Once confluent, cells can be subcultured at 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell line constructed by Dr. DeFilippis. 2.THF-IFIT1, THF-IFIT2, THF-STING, THF-IPS1, THF-IPS1/STING, THF-TRIF, THF-IFNAR, THF-STAT1: Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These cells are also stably transduced with a firefly luciferase-coding region under the control of the interferon responsive element using a lentivector obtained from System Biosciences. Individual cell lines were constructed in which the protein coding regions for IFIT1, IFIT2, TRIF, IFNAR, STING, IPS1, IPS1/STING, or STAT1 were disrupted using the CRISPR/Cas9 system (AddGene vector # 52961). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 106 cells per vial. Cryopreserved cells can be brought up directly into a T75 + 14 mL media. Once confluent, cells can be subcultured at 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell lines constructed by Dr. DeFilippis. 3.CHIKV Caribbean Strain Infectious Clone: CHIKV99659 was recently isolat

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Daniel		Streblow		Sub PD/PI	(b)(4); (b)(6)				13,700.00	4,658.00	18,358.00
2.	Dr	Michael		Axhelm		Co-Investigator					9,255.00	2,314.00	11,569.00
3.	Dr	Victor		DeFilippis		Co-Investigator					11,801.00	4,131.00	15,932.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **45,859.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			9,616.00	3,654.00	13,270.00
	Graduate Students						
1	Undergraduate Students				27,042.00	0.00	27,042.00
	Secretarial/Clerical						
4	Res. Assoc., Microsurgeon, Lab Aide, SPF Proj Mgr				52,850.00	18,828.00	71,678.00
6	**Total Number Other Personnel**					**Total Other Personnel**	**111,990.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**157,849.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		10,000.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**10,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	35,440.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Costs: Set-up fees, Lease fees and Per Diems	61,342.00
9. Other Expenses	31,569.00
Total Other Direct Costs	**128,351.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**299,200.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified Total Direct Cost	75.0	289,200.00	216,900.00
		Total Indirect Costs	**216,900.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Arif M. Karim, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**516,100.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget_Justification_Yr4_WhitleyU19_OHSU_Streblow_Proj3BCM.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION, YEAR 4

Streblow/DeFilippis (Project 3B)

PERSONNEL:

Daniel Streblow, Ph.D., Co-Investigator, years 1-5: (b)(4) months, (b)(4) Dr. Streblow will serve as the OHSU Subcontract PI of Project 3. Dr. Streblow has extensive experience with animal models of infectious disease and has recently teamed up with Dr. Axthelm to develop a non-human primate model of Chikungunya virus infection and disease. His duties will include maintaining active protocols and animal records, coordinating the animal requirements for this Project, facilitating animal experiments, analyzing samples as well as assembling and disseminating data sets produced during this Project. He will ensure timely completion of the proposed work.

Victor DeFilippis, Ph.D., Co-Investigator, years 1-5: (b)(4) months, (b)(4) Dr. DeFilippis will be responsible for *in vitro* experimentation, communicating scientific progress, supervision and training of junior staff, overall experimental design and coordination of drug treatment experiments and determination of mode of action of the antiviral compounds. He is also responsible for data analysis, preparation of reports and publications derived from this part of the Project, as well as communication of research results to the scientific community.

Michael Axthelm, Ph.D., Co-Investigator, years 4-5: (b)(4) months (b)(4) Dr. Axthelm is a veterinary pathologist and an infectious disease specialist with over 20 years of experience investigating mechanisms of viral pathogenesis in non-human primate models, primarily chronic lentivirus and herpesvirus infections. He heads the Infectious Disease Resource that manages the Oregon National Primate Research Center's non-human primate infectious disease protocols. He will advise Al Legasse with respect to coordinating animal selection, protocol implementation, phasing of animal cohorts into the study, and sample and clinical data acquisition. Dr. Axthelm will also advise Mr. Turner in technical aspects of the Project when necessary, including animal sampling procedures, health assessment and anatomic pathology.

Craig Kreklywich, Research Associate, year 4: (b)(4) months, (b)(4) He will be responsible for performing quantitative RT-PCR detection of CHIKV in plasma and tissue samples. He will aid the vet team during necropsy. He is involved in immunohistochemical analysis of CHIKV in tissue samples.

Takeshi Ando, M.D., Microsurgeon, year 4: (b)(4) months, (b)(4) Dr. Ando is a microsurgeon who has been trained in BSL-2 and BSL-3 virological, molecular biological, and animal work. Dr. Ando will be responsible for assisting Dr. Streblow and will be the primary scientist involved in coordinating, conducting, and processing all *in vivo* experiments involving CHIKV.

Michael Denton, Senior Research Assistant, year 4: (b)(4) months, (b)(4) He will be responsible for producing CHIKV titered stocks for infection studies, processing of animal samples, performing titration, CHIKV detection and flow cytometric experiments.

Alfred Legasse, SPF Project Manager, years 4-5: (b)(4) months, (b)(4) Mr. Legasse is the Infectious Disease Resource project manager and has over 25 years of experience working with rhesus macaques as a technician, supervisor and project manager. He will be responsible for scheduling and coordinating day-to-day project activities with the Division of Comparative Medicine animal care staff. He will provide quality assurance for staff training and adherence to standard operating procedures developed to maintain personnel and animal safety and the integrity of the study.

Sara Botto, Postdoctoral Scholar, year 4 (b)(4) months (b)(4) Dr. Botto will work with Dr. DeFilippis in the molecular biological, and animal work. She will be involved in coordinating, conducting, and processing all in vivo experiments.

Rebecca Broeckel, Graduate Research Assistant, year 4: (b)(4) months (b)(4) Ms. Broeckel will work with Drs. Botto and Defilippis in coordination of drug treatment experiments and general lab protocols for this project.

SUPPLIES (Non-Animal Laboratory)

Antibodies ($2,000/year, years 1-2, $5,558/year, years 3-5)
These are necessary for: 1) Detection of viral replication *in vitro* and for immunohistochemistry; 2) Intracellular cytokine staining assays; 3) Flow cytometry for phenotypic analysis of immune responses to viral infection; and 4) Validation of mode of action studies.

Plasticware/Virus Detection ($47,000/year, years 1-3, $8,500/year, years 4-5)
Disposable plasticware will be required for cell and virus culture, CHIKV titration and virus isolation, and molecular biological work. This includes tissue culture dishes of myriad sizes and layouts, flasks, serological pipettes, disposable pipette tips, microfuge and centrifuge tubes, and disposable screw cap tubes of various sizes for sample storage. This also includes virus detection reagents for quantitative PCR (e.g., Taq polymerase, primers, TaqMan probes, 96-well plates).

Tissue Culture Supplies ($37,200/year, years 1-3, $8,500/year, years 4-5)
These will be required for all cell growth and maintenance as well as virus growth and titration and isolation from tissues. This includes cell culture growth media, animal serum, PBS, trypsin, sucrose, sorbitol, disposable sterilizing filters, antibiotics, and syringes.

Virus Detection Supplies ($20,000/year, years 1-3, $10,000/year, years 4-5)
qRT-PCR will be used for the detection of both CHIKV and DENV. Reagents for virus detection include: Reverse transcription reagents, ABI Master mix containing Taq polymerase, virus-specific primers and TaqMan probes, 96-well optical plates.

LN_2 and CO_2 ($2,882/year, years 3-5)
Liquid nitrogen and carbon dioxide will be needed for incubators, cryopreservation, enzymes for tissue digestion, and tissue fixatives.

ANIMAL COSTS:
Largely fees assessed for animal maintenance (per diem), and fees for surgical services provided by the ONPRC Division of Comparative Medicine staff. The experiments described for years 1-3 were designed to test larger numbers of compounds (or refined compounds) in a mouse model of CHIKV infection and disease. However, in years 4-5 we will test 1-2 of the best candidate compounds in a Rhesus macaque model of CHIKV infection and disease. The prices per year reflect the experimental design.

The number and cost of each of these items are provided in the following other expenses summary:

Estimated number of NHP: Years 4-5, 8 animals.

Rhesus macaque lease fees ($53,902/year, years 4-5 only)
Rhesus macaques cost $6,737.76/animal between the ages of 5-11 years old. The lease fees reflect the portion of the true production costs, and are standardized for all Public Health Service grantees using the ONPRC. Years 4-5: 8 animals x 6,737.76 = $53,902 per year.

Rhesus macaque set-up fees ($1,547/year, years 4-5 only)
$193.41/animal, are charged by the Division of Comparative Medicine to defray the administrative costs of animal selection, records requirements for assignment and initial health assessment to insure healthy animals are assigned to projects. Years 4-5: 8 animals x $183 = $1,547 per year.

Rhesus macaque per diem, ABSL 3 ($5,892/year, years 4-5 only)
$52.61/animal/day for 14 days for 8 monkeys per year in FY4&5.

OTHER EXPENSES:

Necropsy fees ($17,291/year, years 4-5 only)
$2,161.43/animal, 8 animals/year for Years 4 and 5 only (Grade 3 Complex necropsy & histopathology).

Flow cytometry charges ($3,000/year, years 1-5)
We are charged $60/hour of FCM time, which will be used to analyze peripheral blood samples for specific cellular markers as well as when performing intracellular cytokine staining assays for NHPs. We are estimating 50 hours of FCM time per year.

Veterinary time (physical exams) ($3,282/year, years 1-5)
$32.82/animal/hour. We expect roughly 100h total vet time per year. Includes analysis of unexpected complications arising from dugs/infections in mice and NHP.

Slide Preparation and Histology ($3,995/year, years 1-5)
We are charged $5.58 for processing of tissue samples, cutting and mounting, H&E staining. We are estimating that we will need $3,995/year for slide preparation and staining.

Sample collection and drug and agent administration (Surgical Supplies) ($1,000/year, years 1-3; $2,000/year, years 4-5)
Vacutainer blood tubes, needles, syringes and sterile plastic collection tubes and swabs required for obtaining blood samples and tissues from both NHP and mice.

Equipment Maintenance ($2,000/year, years 3-5)
This proposal will require the use of our general laboratory equipment, which must be maintained to properly execute this study. Therefore, we are requesting $3,177 in years 3-5 to maintain the equipment in good working order.

Tuition and Fees ($10,000/year 4)
We request $10,000 in tuition and fees, per OHSU graduate student rates, for Ms. Broeckel.

TRAVEL:

DomesticTravel ($3,000/year, years 1-5)
$3,000/year for Co-Investigators to attend an international meeting pertaining to antiviral therapeutics directed against emerging RNA viruses.

A. COMPONENT COVER PAGE

Project Title: Project 4.1 Identification and characterization of novel drugs that target the Influenza virus polymerase functions

Component Project Lead Information:

Whitley, Richard J.

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific aims: The overall goal of this project is to identify new therapies that target influenza virus replication. The global health burden of annual influenza epidemics coupled with the emergence of highly pathogenic strains of influenza virus has highlighted the urgent need for new effective treatments. A primary concern with the current drugs (amantadines and neuraminidase inhibitors) used to treat influenza is the development of resistance mutations that negate therapeutic benefit. Published evidence suggests that targeting the influenza virus RNA dependent RNA polymerase (RdRp) is a rational approach for antiviral therapy. The RdRp is responsible for a number of functions including 5'cap recognition, endonuclease activity, replication, transcription, and polyadenylation. Recently, cryo-EM reconstitution studies identified branched-ribonucleoproteins (RNPs) structures as putative replication intermediates and suggested a mechanism for viral replication by a second polymerase activity on the RNP template [1]. The second polymerase activity is believed to be a function of the polymerase complex. Clearly, the RdRp provides multiple functional domains that could be targets for antiviral drug therapy. Previous studies showed that mutations in the conserved regions of PB1 subunit of the polymerase complex produce inactive RNA polymerase [2]. We hypothesize that compounds that specifically target the polymerase complex might reduce the frequency of escape mutations, or promote escape mutants that are unfit for replication. We have recently identified potential hit compounds from previous HTS screens that significantly inhibit the influenza virus polymerase activity in an RdRp transient assay. These hit compounds were effective against three different strains of influenza viruses in CPE assays. Between Southern Research (SR) and the University of Alabama at Birmingham (UAB), all the necessary primary and secondary assays to perform HTS screening and identify compounds that specifically target the influenza virus polymerase activity have been developed. We propose the following specific aims:

Aim#1. Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block influenza virus replication.
Hypothesis and rationale: We hypothesize that by targeting the polymerase complex, we might reduce the frequency of mutational evasion because the mutants will be unfit for replication. Recent studies demonstrated that the nucleoside inhibitor T-705 induces lethal mutagenesis in H1N1 viruses in vitro resulting in a nonviable phenotype [3]. Targeting the influenza polymerase activity might prove more effective than targeting the viral glycoproteins because there are multiple proteins, as well as protein: protein and protein: RNA interactions, which could be targeted. Our goal is to identify compounds against the conserved regions of influenza virus polymerase subunits that might be effective against multiple viral strains.
Experimental strategy: The proposed transient influenza polymerase assay in aim#2 to identify anti-polymerase hits is not adaptable for HTS, and therefore a CPE-based assay will be used as a primary assay to screen novel libraries against influenza viruses. We will screen libraries that have not been previously screened for activity against the viruses covered in this proposal. These libraries are composed of highly diversified small molecules that contain novel and original drug-like features with distinct topologies and diverse functionalities.

Aim#2: Characterize the antiviral activity of hit compounds and identify anti-polymerase inhibitors.
Hypothesis and rationale: The existing hit compounds with polymerase inhibitory activity might target one or more subunits of the influenza virus polymerase. The CPE-based HTS screening will identify additional hit compounds that target all stages of the virus life cycle, including multiple functional domains of the influenza RNA polymerase. We have designed an experimental strategy that will focus our analysis on the hit compounds that block post-entry steps of viral infection.
Experimental strategy: We will use a variety of secondary assays to identify compounds that specifically inhibit the functions of the viral polymerase complex. Our proposed secondary assays will identify and exclude hit compounds that target viral entry and release, as well as interferon inducers. Following this exclusion process we will examine the remaining positive hit compounds in the transient polymerase assay. Once compound specificity for the viral polymerase is demonstrated, tertiary assays will be performed to determine the target within the polymerase complex.

Aim#3: Chemical optimization and determination of the in vivo efficacy of lead compounds.
Hypothesis and Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit the influenza replication complex. Chemical optimization of the effective scaffolds should generate compounds with greater efficacy, selectivity, and bioavailability.
Experimental strategy: The hit compounds from the HTS will be triaged and progressed as outlined in the Chemistry core. Compounds with the appropriate activity and pharmacokinetic properties will be evaluated using in-house mouse infection models.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Project 4.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

As noted elsewhere, the HTS core completed the primary screen, and 892 hits were identified. The hits were evaluated at concentrations of 10 and 2 μM in a CPE-based assay in MDCK cells for activity against A/CA/10/2009 (H1N1) and A/Panama/2001/99 (H3N2), as well as for cytotoxicity at the same concentrations. The data are currently being analyzed. Confirmed hits from this analysis will be evaluated for potency and efficacy by determining the EC50 values in the MDCK-based CPE assay, and the extent of virus titer reduction (VTR) in the MDCK cells. Compounds active against both subtypes (EC50 ≤20 μM, CC50 ≥50, and VTR of ≥2-log10), will then be evaluated in the RNA-dependent RNA polymerase (RdRp) assay to determine if the activity is due to inhibition of the polymerase. Each compound will be tested at concentrations ranging from 0.39-50 μM, and the compounds with EC50 ≤20 μM in the RdRp assay will be selected for further analysis and preliminary structure-activity relationship studies. This will involve iterative cycles of compound design and synthesis of analogs, and their evaluation in MDCK-based CPE and VTR assays. The activity of promising compounds will then be tested in the primary human small airway epithelial cells using the NanoLuc influenza PATSN (H1N1), as well as against the avian (H5N1) subtype in MDCK cells. To identify the drug target for the active compounds, resistant mutants against each will be generated. The entire genomic RNA segments encoding the different polymerase subunits will be sequenced for each mutant to identify the precise drug target sites within the polymerase complex. In the unlikely event that no polymerase inhibitor could be identified, all active compounds (EC50 ≤20 μM, CC50 ≥50, and VTR of ≥2-log10 in the MDCK-based CPE and VTR assays) will be tested in Neuraminidase inhibition, Hemagglutination inhibition, and Virus Entry assays to identify their possible mode of action. Alternatively or additionally, resistant mutants will be generated and the entire genome will be sequenced for each mutant to identify the other potential viral targets.
With respect to future in vivo studies, The in vivo team purchased and documented accuracy of a microrectal thermometer for measuring body temperatures of mice as required by IACUC for influenza studies. Based on availability of nanoluc technology, we modified our regulatory files with IACUC and IBC to include imaging of infected mice through the UAB Imaging Core directed by Dr. Kurt Zinn. Once approved, mice will be purchased for lethality studies to determine the optimal dose for viral intranasal infections in BALB/c mice. Within weeks of the pilot study, we'll be prepared to initiate efficacy and toxicity studies of lead compounds.

B.2. Accomplishments under these goals:

B2.1. Evaluation of chemical analogs of the compound SRI 34518.

In the Year 2 report, we provided data indicating that SRI 34518, exhibited inhibitory activity against the pandemic (A/California/07/2009, H1N1), seasonal (A/Udorn/72, H3N2) and highly pathogenic avian (H5N1) subtypes of Influenza A virus (IAV) with EC_{50} values of 13.9, 5.03, and 7.01 µM, respectively. The compound was also found to be active in the minigenome-based IAV RdRp (polymerase) assay, whereby it inhibited the activity of IAV RdRp with an EC_{50} of ~3.0 µM. These data suggested that SRI 34518 is likely an IAV polymerase inhibitor with activity against multiple subtypes. However, structurally it does not provide a viable starting point. Therefore, 23 analogs of SRI 34518 were obtained and tested in the ELVIRA (reporter cell line) assay against the pandemic (H1N1) and seasonal (H3N2) IAV subtypes. Of these, only three compounds (SRI34993, SRI 34997, and SRI35058) were found to be active against both IAV subtypes with an EC_{50} <20 µM. Of these three, only SRI 34993 exhibited a lower EC_{50} and higher selectivity index (SI) than the parental compound SRI 34518. The compound SRI 34993 was then tested in the IAV minigenome RdRp assay and found to be inactive, indicating that SRI 34993 does not target the IAV polymerase; demonstrating that its anti-IAV activity is through a distinct mechanism. In addition, SRI 34993 was also tested against the avian (H5N1) IAV in then MDCK-based CPE assay and found to be inactive. Since the compounds tested here either had lower activity than the parental compound or did no inhibit the IAV polymerase, further work on this series is on hold at this time.

B2.2. Evaluation of compounds from Projects 1-3

One of the goals of the U19 is to identify potential targets or mechanisms with more broad applications. Too that end, a set of 18 compounds that exhibited activity against the viruses screened in Projects 1-3 were selected for screening as part of project 4. The compounds were tested against A/Udorn/72 (H3N2) subtype in the ELVIRA reporter cell line in a concentration response assay. Seven compounds (SRI 27298, 33361, 35894, 36418, 36422, 3678, 36772) inhibited IAV replication with an EC_{50} of <10 µM, and three compounds (SRI 35756, 36771, 36770) with an EC_{50} between 10 and 20 µM. Subsequently five compounds (SRI 33361, 36418, 36422, 36768, 36772) that had EC_{50} ≤5.0 µM in the ELVIRA assay were tested in the IAV RdRp assay to determine if they inhibited the IAV polymerase. Each compound was tested at concentrations ranging from 0.39 – 50.0 µM. All were active at (<3.0 - <6.0 µM) except SRI 36772. The compounds were also tested for their cytotoxicity 24 h and 72 h post-treatment in three different cell types including HEK293, A549, and MDCK. None of the compounds had any significant cytotoxicity 24 h post-treatment; however, SRI 33361 and SRI 36422 were cytotoxic in HEK293 cells with CC_{50} values of 2.66 and 2.21 µM, respectively, 72 h post-treatment. All 18 compounds were also evaluated in a CPE assay in MDCK cells against A/CA/10/2009 (H1N1), A/Panama/200I/99 (H3N2), and B/Florida/4/2006, using a concentration range of 0.016 – 50 µM. However, none of the compounds were found to active against Influenza A virus, and only three, SRI 35894, SRI 36768, and SRI 36770, displayed modest antiviral activity against the B strain of influenza virus with (EC_{50} values of 7.9 ± 12, 5.1 ± 5.9, and 10.2 ± 12.9 µM, respectively, and CC_{50} values that ranged between 21 and 24 µM). Therefore, due to inactivity against Influenza A virus and/or high cytotoxicity, no follow up work is planned for these compounds.

B2.3. Confirmation of antiviral activity of the 892 active compounds from the HTS screen.

The HTS core identified 892 compounds with confirmed antiviral activity in a dose response assay utilizing the ELVIRA reporter cell line. After Clustering and PANE filtration, these compounds were evaluated further in a 384-well CPE assay in MDCK cells at concentrations of 2 and 10 µM against A/CA/10/2009 and A/Panama/200I/99, and a cytotoxicity assay was completed with equivalent compound exposure. After data analyses, the top 14 hits that displayed >50% antiviral activity against both strains of Influenza A virus with <20% cytotoxicity at 10 µM have been selected for further analyses, as detailed in the plans for Year 4.

B2.4. Development of a 384-well influenza assay in primary small airway epithelial cells.

An assay was developed in 384-well plates using primary human small airway epithelial cells and the NanoLuc influenza strain A/California/04/2009 pdm (H1N1) PATSN. While this strain does not exhibit CPE in these cells, the NanoLuc activity expressed over two rounds of replication provided a reliable indicator of virus replication in these cells. This assay will prove to be valuable in downstream studies as it will allow further evaluation and confirmation of the lead compounds in primary human lung cells representing a more physiologically-relevant model system.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Richard	J	Whitley		PD/PI	(b)(4); (b)(6)				18,510.00	5,701.00	24,211.00
2.	Dr	Mark		Prichard		Co-Investigator					13,881.00	4,275.00	18,156.00
3.	Dr	Debra		Quenelle		Co-Investigator					12,255.00	3,774.00	16,029.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **58,396.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	1 Research Supervisor, 3 Research Technicians	(b)(4)			22,838.00	7,788.00	30,626.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**30,626.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**89,022.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,910.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Per Diem	2,776.00
9. Sequencing, Other Services	4,250.00
10. Publication, Copying/Printing, Shipping	1,000.00
Total Other Direct Costs	**23,936.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**115,958.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	115,959.00	54,501.00
		Total Indirect Costs	**54,501.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**170,459.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Year 4 Budget justification Proj 4.1 SD 12.2.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Program Director/Principal Investigator (Last, First, Middle): Whitley, Richard J. (Project 4.1)

Budget Justification

Personnel

Richard J. Whitley, MD, PD/P (b)(4) months: Dr. Whitley will continue to serve as the UAB Program Director/Principal Investigator for Project 4 investigating the inhibitors of influenza virus. He continues to provide broad oversight of the project and serves as primary liaison with the External Advisory Board, NIH and other external entities, including pharmaceutical companies to determine potential compounds to be developed, and international groups such as the IDSA to determine therapeutic needs.

Mark Prichard, PhD (b)(4) months: Dr. Prichard has conducted research in discovery and development of antiviral drugs for more than twenty years. He continues as the PI of an NIAID contract focused on the evaluation of compounds for antiviral activity against the human herpesviruses and the orthopoxviruses. He continues to work closely with Southern Research on development of assays and evaluation of compounds from the HTS work that has just been completed. He has also been working with the in vivo group on determining compounds for use in animal models and with the testing of drug resistance.

Debra Quenelle, DVM, PhD (b)(4) months: Dr. Quenelle has more than 27 years of experience in use of animal models in infectious diseases, and is the PI for an NIAID contract to test compounds in animal models. Preliminary animal work, which was begun this year, will continue in Year 4. Dr. Quenelle will oversee the in vivo studies in mice to determine the antiviral activity and toxicity of the compounds identified in the compound screening.

Kathy Keith, MS, Laboratory Supervisor (b)(4) months,: Ms. Keith more than 25 years of experience in laboratory work on a number of viruses including HIV, α&β-herpes, influenza, vaccinia and cowpox primarily determining in vitro drug efficacy using different endpoint methods (e.g., ELISA, CPE, plaque reduction, virus yield, hybridization, real time PCR) and under Biosafety levels 2 - 3. She will continue to oversee the antiviral assays and day to day activities for this project in Dr. Prichard's laboratory.

Deborah Collins, Research Technician (b)(4) months, Ms. Collins has more than 20 years of experience working with experimental animal studies, both large and small animals. She will continue to assist with the mice studies which are beginning for the project

Terri Rice, Research Technician (b)(4) months, : Ms. Rice has 18 years of prior experience with small animal toxicology and pharmacology studies. She will continue to assist with all animal studies performed as part of the project.

Jessica Eagar, Research Technician (b)(4) months,: Ms. Eagar has more than 4 years of experience in research labs. She will provide general assistance with assays and in vitro lab work.

Supplies

Funds are requested for tissue culture, reagents, surgical supplies, PPE, labware and miscellaneous laboratory supplies need to conduct the planned compound testing. In addition, in funds are included to provide for purchase of mice to be used in the in vivo testing.

Travel

Funds are requested to allow travel of the PD/PI and co-investigators to attend CETR or related scientific meetings to present results of the study.

Other Expenses

Funds are requested for shipping of materials, sequencing, maintenance of project specific equipment, and publication costs. Costs are also requested to cover per diem costs for care of mice being used in the studies.

A. COMPONENT COVER PAGE

Project Title: Screening Core - Core B

Component Project Lead Information:

(b)(6); (b)(3):7 U.S.C. § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

B1: Major goals

The overarching goal of the Screening Core (SC) is to identify chemical series with anti-viral effects in high throughput screens against multiple virus targets and to assist in converting them into drugs by providing in vitro biological screening support to the Medicinal Chemistry and Lead Development Core (MCLDC). By screening a unique, common compound collection against each virus, the screening core seeks to identify selective as well as broad-based inhibitors of viral replication in accordance with the theme of the program.

Specific Aims
Aim 1: Identify hit compounds for influenza, dengue, Venezuelan equine encephalitis, West Nile, Chikungunya viruses, and SARS Coronavirus. The overall aim of the SC is to identify hit compounds that inhibit replication of influenza (INFV), dengue (DENV), Venezuelan equine encephalitis (VEEV), West Nile (WNV), Chikungunya (CHIKV), and/or SARS CoV. A cytopathic effect (CPE) assay will be employed for screening against VEEV, WNV, CHIKV, DENV and SARS CoV. Different assay readouts will be investigated for screening INFV, including a reporter gene and viral titer assays. The CPE assay for SARS CoV will be run in multiple conditions to identify inhibitors of virus replication by unknown mechanisms of action as well as those specifically targeting CoV fidelity and RNA capping. Each of the viruses will be screened using the same 300,000 member library that was selected due to its unique properties with regards to chemical diversity, drug-like properties and potential ability to modulate a variety of biological pathways and targets involved with viral replication. By using a common library for all of the assays, compounds that are active across several viruses may be identified and could result in the identification of targets with broad spectrum activity. Primarily this will be accomplished by sharing the compounds among the consortium participants and by establishing an Antiviral Drug Discovery and Development Consortium (AD3C) database using Enterprise Content Management Documentum CenterStage, where all of the assay conditions and screening results will be uploaded. All the Consortium participants have access to this secured site.
Aim 2: Perform the assay(s) for each virus to be used to provide the biological support for each virus for structure-activity studies by the Medicinal chemistry and Lead Development Core (MCLDC). As the hits from the HTS assays are developed, a moderate throughput assay is needed for each target to quantify the changes in activity that occurs as structural modifications are made to the hit compounds. These assays (SAR driving assays) will be used in the design-make-test cycle to determine structure-activity relationships that will be important for developing lead series and compounds suitable for in vivo testing. As additional mechanistic studies are completed by the various groups, supplemental cell based or biochemical assays may be incorporated into the project.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Core B 2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B.6. Plans for next reporting period
Aim 1: The HTS campaign for Zika will be initiated and completed. We will screen the same 300K compound collection as was done for Dengue and SARS.
Aim 2: The SC will conduct the anti-viral assays relevant for each project (listed in Table 2 in the PDF uploaded under section B2) to determine IC50 values for newly synthesized compounds produced by the MCLDC. This work will be an integral component of the iterative design-make-test cycle during the lead generation phase of these projects.

B2: Accomplishments under these goals

Aim 1 has been accomplished with the completion of HTS campaigns for all six virus targets (summarized in Table 1). Details for each project are described in Core Specific information below.

Supplemental funds were awarded in August 2016 (midway through grant year 3) to conduct an HTS campaign for the Zika virus (ZIKV). This work has been initiated. Zika Paraiba stock obtained from the Diamond lab has been propagated in C6/36 mosquito cells. Vero-CCL 81 cells show adequate CPE five days after being infected with this virus stock (> 80% cytopathic effect). The assay has been optimized with respect to cell plating density, virus stock dilution for infection and days post infection to measure CPE. The optimized assay has been validated for use in HTS by screening a collection of 5,000 biologically active compounds (including a set of FDA approved drugs) twice on separate days. The average signal/background ratio for the control wells (viability of uninfected cells / viability of virus infected cells) was 4.7 and the average Z' value was 0.6. These values demonstrate that the assay is sufficiently robust for HTS. Interestingly, the screen reproducibly detected antiviral activity of pirodavir, a broad spectrum rhinovirus entry inhibitor. A fresh supply of this compound tested at 10 concentrations showed an IC_{50} value of ~50 µM in this assay. The HTS campaign will be run using this compound as a reference.

Table 1. Summary of HTS campaigns specified in Aim 1.

Target	Assay	Virus strain	host cell	# compounds screened	# preliminary hits	# validated hits	grant year completed
DENV	CPE	New Guinea (VR-1584)	HEK 293	304,810	2,240	45	Year 1
WNV	CPE	NY99	HEK 293	197,077	2,997	160	Year 3
SARS	CPE	Toronto	Vero E6	305,648	2,492	575	Year 1
CHIKV	CPE	Sri Lanka	Vero E6	197,025	2,558	44	Year 2
VEEV	CPE	TC-83	Vero E6	197,025	940	42	Year 2
INFV	Reporter	H3N2 (Udorn)	HEK 293	196,721	3,200	1,197	Year 3

Aim 2 has been partially accomplished. The SAR driving assays for each virus target have been developed (summarized in Table 2). Their use to support chemistry efforts is ongoing.

Table 2. Summary of SAR driving assays

Target	host cell	Assay technique	Assay measure	Readout
DENV	HEK 293	immunofluorescence	viral protein expression	fluorescence
WNV	HEK 293	CPE	host cell viability	luminescence
SARS	Vero E6	virus reporter	nanoluc enzyme activity	luminescence
CHIKV	Teleomerized human fibroblasts	virus reporter	nanoluc enzyme activity	fluorescence
VEEV	Teleomerized human fibroblasts	CPE	host cell viability	luminescence
FLUV	HEK 293	host cell reporter	luciferase enzyme activity	luminescence

Core specific information

Project 1. Flaviviruses

Dengue virus

Aim 1 (accomplished Year 1): A CPE assay employing a dengue viral stock prepared in insect cells and HEK293 host cells was used to screen a total of 304,810 compound samples. Using an activity threshold of inhibition ≥ 26.25% (mean + 3xSD of all data), 2,240 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Forty-five (45) compounds were confirmed and validated as hits with $IC_{50} < 20$ µM and no cytotoxicity. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An immunofluorescence assay measuring viral protein expression in the host cell was developed and is being used to develop SAR for hit-to-lead and lead optimization chemistry efforts.

West Nile Virus

Aim 1 (accomplished Year 3): A CPE assay was constructed to identify inhibitors of the viral 2'-O-Methyltransferase. The 2'-O-MTase activity of flaviviruses promotes viral evasion of the Ifit family of genes, a group of host cell IFN-stimulated immune effector proteins. In order to detect inhibitors of virus 2'-O-MTase activity, the HTS assay was performed using transformed HEK 293 cells that expressed Ifit1 when induced by doxycycline. Such compounds will promote the host cell defense mechanism and reduce CPE. The assay also detected compounds that had a direct anti-viral effect since those compounds reduced CPE independently of Ifit expression. A total of 197,077 compounds were screened using HEK cells treated with doxycycline to induce ifit1 expression. Using a statistical threshold of inhibition ≥ 19.03% (mean + 3xSD of all data), 2997 compounds were identified as active. In order to confirm hits and distinguish potential inhibitors of 2'-O-MTase activity from those with direct anti-viral activity, the compounds were retested at 10 concentrations for inhibition of CPE and direct cytotoxicity effects in HEK cells treated with or without doxycycline (i.e. with or without ifit1 expression). IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Hits were deemed confirmed and valid if they had an $IC_{50} < 75$ µM and no cytotoxic effect. By this criteria, 30 compounds were active only if ifit1 was expressed (i.e. active only in cells treated with doxycycline) and were identified as potential inhibitors of the viral 2'-O-Methyltransferase. An additional 130 compounds were active independent of Ifit expression and identified as those having direct anti-viral effects. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): The CPE assay is currently available to develop SAR for hit-to-lead and lead optimization chemistry efforts but an immunofluorescence assay is being developed for future use.

Project 2. SARS Corona Virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells selected for expression of the SARS CoV receptor (ACE2; angiotensin-converting enzyme 2) were used to screen a total of 305,648 compound samples. Using an activity threshold of inhibition ≥ 80%, 2,492 samples

were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Of these, 307 compounds were confirmed and validated as hits showing IC_{50} < 20 µM and SI (IC_{50}/CC_{50}) > 3. An additional 268 compounds were confirmed and validated as hits showing IC_{50} > 20 µM and SI (IC_{50}/CC_{50}) > 3. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An assay measuring reporter luminescence as an index of virus titer was developed using a recombinant SARS Nanoluc virus produced in the Baric lab. The assay is employed to measure the anti-viral effects of newly synthesized compounds to support development of SAR for hit-to-lead and lead optimization chemistry efforts.

Project 3. Alpha Viruses

Chickungunya virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells were used to screen a total of 197,025 compound samples. Using an activity threshold of inhibition ≥ 50.38% (mean + 3xSD of all data), 2,558 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects using Teleomerized Human Fibroblast (THF) cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. Forty-four (44) hits were confirmed and validated with IC_{50} < 20 µM and SI (IC_{50}/CC_{50}) > 10. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An assay measuring reporter luminescence as an index of virus titer was developed using a recombinant CHIKV Nanoluc virus produced in the (b)(6); (b)(3):7 lab. The assay is employed to measure the anti-viral effects of newly synthesized compounds to support development of SAR for hit-to-lead and lead optimization chemistry efforts.

Venezuelan Equine Encephalitis virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells were used to screen a total of 197,025 compound samples. Using an activity threshold of inhibition ≥ 12.12% (mean + 3xSD of all data), 940 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects using Teleomerized Human Fibroblast (THF) cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. Forty-two (42) hits were confirmed and validated with IC_{50} < 20 µM and SI (IC_{50}/CC_{50}) > 10. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): The CPE assay is currently available to develop SAR for hit-to-lead and lead optimization chemistry efforts. A virus titer reduction assay has also been developed to use in conjunction with the CPE assay data.

Project 4. Influenza A viruses

Aim 1 (accomplished Year 3): An enzyme linked virus inhibitor reporter assay was used for HTS. This assay (described in Lutz et al., J. Virol. Methods 2015, 126: 13-20) utilizes an

HEK293 cell line engineered to express virus-like negative sense RNA transcripts encoding firefly luciferase flanked by the untranslated regions of influenza A/WSN/33 NP segment. (ELVIRA® Flu A-luc cells). When these cells are infected by influenza A, the virus RdRp transcribes this RNA into mRNA and luciferase protein is produced. Luciferase enzyme activity is then measured as a reporter of virus infection enabling the anti-viral activity of test compounds to be determined by a decrease in luciferase activity. A total of 196,721 unique compounds were screened in HTS. Using an activity threshold of inhibition ≥ 83.45% (mean + 3xSD of all data), 3200 samples were identified as active and retested at 10 concentrations for anti-viral and direct cytotoxicity effects. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. A total of 1197 hits were confirmed and validated showing $IC_{50} < 20$ µM and SI (IC_{50}/CC_{50}) > 10 in the ELVIRA® Flu A-luc HEK reporter cells. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): The reporter assay used for HTS can also be employed to measure the anti-viral effects of newly synthesized compounds to support development of SAR for hit-to-lead and lead optimization chemistry efforts.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

Section F: Changes
As stated in the year 2 update, the screening collection was reduced to ~222K for WNV and FLUV by omitting the Chembridge Set 3 to stay within budget. The collection will be expanded back to ~315K compounds by re-including the Chembridge Set 3 for the ZIKV HTS campaign since adequate supplemental funds were awarded.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

File uploaded: Core B F 3.pdf

F.3: Significant changes to select agents

Following CDC approval, the recombinant SARS CoV (Urbani strain) expressing a NanoLuc reporter was transferred to (b)(6); (b)(3):7 U.S.C. § 8401 This virus is currently being used in assays to evaluate newly synthesized compounds for development of SAR.

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-8279

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1		(b)(6); (b)(3):7 U.S.C. § 8401				Project Leader	(b)(4); (b)(6)				32,926.00	15,344.00	48,270.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **48,270.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
11	1 Supervisor Comp. Mgmt., 4 Biologist, 3 Informatics, 1 Supervisor HTS Ctr., 1 Scientist, 1 Project Mgmt.	(b)(4)			134,582.00	62,717.00	197,299.00
11	**Total Number Other Personnel**					**Total Other Personnel**	**197,299.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**245,569.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,500.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**2,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	321,486.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Disposal of HazMat	53,280.00
Total Other Direct Costs	**374,766.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**622,835.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH-Salaries and Benefits	120.0	245,569.00	294,683.00
2. G&A-Total Direct Cost + OH	20.0	917,518.00	183,504.00
3. CFC-Salaries + Benefits	7.3	245,569.00	17,927.00
4. CFC-Total Direct Cost + OH	1.0	917,518.00	917.00
		Total Indirect Costs	**497,031.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Steven Zuraf, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,119,866.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Year 4 Justification for Screening Core B.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Year 4 Budget Justification for the Screening Core

(b)(6); (b)(3):7 U.S.C. § 8401 Ph.D. will serve as Project Leader of the screening core. (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 combined experience in academia and the pharmaceutical industry involving work in over 60 drug discovery programs. (b)(6); (b)(3):7 U.S.C. has expertise in the development and use of biochemical and cell-based assays in HTS with a focus on translational relevance to ensure that HTS output can be effectively exploited as part of a comprehensive approach to chemical probe and lead generation. (b)(6); (b)(3):7 will have oversight for the automation and execution of the high throughput screens, counter and specificity screens, and biological support for the SAR studies. In collaboration with the PIs and other Co-Investigators, (b)(6); (b)(3):7 will assist in interpretation of the biological results; report, manuscript and patent preparation; and overall project management. (b)(6); (b)(3):7 will devote (b)(4) (b)(4) months in YR4 to this program.

(b)(6); (b)(3):7 U.S.C. § 8401 M.S., will supervise and manage the day to day efforts for assay development and screening including scheduling, equipment maintenance and QCing, and data QC. (b)(6); (b)(3):7 brings her expertise in keeping the Center with an annual operating budget of over $3,000,000 operating efficiently. (b)(6); (b)(3):7 will devote (b)(4) months in YR4 to the project

(b)(6); (b)(3):7 U.S.C. § 8401 MS, PMP has five years experience in coordinating and managing research projects. (b)(6); (b)(3):7 will work with Dr. Suto and the other project leaders to ensure a timely and efficient delivery of Core services to the overall program and will devote (b)(4) months in YR4 to the program.

HTS Center Personnel – will be responsible for executing the ZIKV HTS assay and the biological assays driving SAR including compound handling and informatics support for data analysis:

(b)(6); (b)(3):7 U.S.C. § 8401 M.S., will be responsible for compound management and drugging for the biological assays. (YR4 (b)(4) months)

(b)(6); (b)(3):7 U.S.C. § 8401 B.S., will assist (b)(6); (b)(3):7 U.S.C. § 8401 drugging for the HTS. (YR4 (b)(4) months)

(b)(6); (b)(3):7 U.S.C. § 8401 B.S., oversees the HTS informatics group and will be responsible for writing the data templates for the screening effort and data import and analysis and depositing the data with Enterprise Content Management Documentum CenterStage database. (b)(6); (b)(3):7 U.S.C. § 8401 manages our ActivityBase software, the Oracle database, and will facilitate transfer of data between the groups including the cheminformatics staff. (YR4 (b)(4) months)

(b)(6); (b)(3):7 U.S.C. § 8401 B.S., will be responsible for importing the data from the plate readers into the analysis software and generating data reports. (YR4 (b)(4) months)

(b)(6); (b)(3):7 U.S.C. § 8401 M.S. will be responsible for the statistical analysis of the high throughput screening data. (YR4 (b)(4) months)

(b)(6); (b)(3):7 U.S.C. § 8401 M.S. will be responsible for running the ZIKV HTS assay and the SAR driving anti-viral assays for all other virus targets. (b)(6); (b)(3):7 U.S.C. § 8401 has BSL3 certification and is trained to work with select agents. (b)(6); (b)(3):7 efforts will include assay automation and data verification. (YR4 (b)(4) months)

(b)(6); (b)(3):7 U.S.C. § 8401 M.S., will assist in the execution of the SAR driving assays requiring work in the BSL-3 and will be responsible for preparing cells, media, reagents, barcoding plates, and reading plates and the execution of the cell cytotoxicity assays. (YR4 (b)(4) months)

(b)(6); (b)(3):7 U.S.C. § 8401 B.S, will provide laboratory operations support including instrument repair and maintenance (YR4 (b)(4) months)

(b)(6); (b)(3):7 U.S.C. § 8401 will be responsible for growing and maintaining cell lines for supplying the biological assays. (YR4 (b)(4) months)

Other Direct Costs: $203,986 in YR4 has been budgeted for the purchase of biochemical supplies and reagents such as tips, microtiter plates, buffers, media, cells and detection reagents (i.e. Cell Titer Glo). Also, $117,500 in YR4 has been budgeted for the HTS service center charge at a rate of $300/hour for robot usage to prepare compound and assay plates and perform automated screening. This charge includes service contracts, depreciation for the automation equipment, and regular QCing of the equipment. A BSL3 facility charge in the amount of $53,280 is also budgeted in YR4 (listed as HazMat charge). There is a travel request of $2500 for (b)(6); (b)(3):7 U.S.C. § 8401 o attend the annual CETR meeting.

A. COMPONENT COVER PAGE

Project Title: Medicinal Chemistry and Lead Development Core - Core C

Component Project Lead Information:

Pathak, Asish

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The primary goal of the MCLDC is to provide hit-to-lead analysis, synthetic chemistry, structure-activity relationship (SAR) data and analysis, computational support, and lead optimization chemistry to further the AD3C's mission of developing new broad-based therapeutics for the treatment of infections caused by emerging pathogens. In this role, the MCLDC, in conjunction with the Screening Core (SC; Core B), will be the central focus of the translational research component of the program. As the SC optimizes the novel assays developed by various Research Projects, and subsequently prosecutes the screening campaign, it will be the function of the MCLDC to assess the quality of the hit compounds that emerge, and ultimately to convert novel, tractable hits into potential clinically useful drugs with optimized biological and biophysical properties.

The Specific Aims of Core C, which remain unchanged, are:

Aim 1: Optimize screening hits identified through the primary HTS, dose-response, secondary assays, and counter screens to identify compounds with the activity, selectivity, and pharmacokinetic properties to warrant animal testing. For example, a typical compound that meets these criteria will have free plasma concentrations in the mouse (or rat, when administered IP, SC or PO) that exceeds the EC50 of the compound's primary activity by 2 to 5 fold for a period of time to be determined by the in vivo model used and the associated in vitro data. The MCLDC will be responsible for all phases of optimization, scale-up, and submission to the SC for testing in the primary SAR screen as well as providing samples to the Center's participants. All newly synthesized compounds will be fully characterized using standard spectroscopic and chromatographic tools (HPLC, LC/MS, NMR, MS, and elemental analysis as appropriate). In addition, the MCLDC will be responsible for performing a freedom to operate analysis as well as coordinating the filing of patent applications relating to new compounds when appropriate.

Aim 2: Provide integrated informatics support including compound tracking, data capture, data analysis, and data storage, backup, and retrieval. For each assay, an appropriate Protocol ID will be assigned to track data relating to the informatics operations. For each compound synthesized, we will import structures and assign a unique identifier (via our in-house Dotmatics registration database). This identifier (or number) will be used throughout the Center to track compounds and any associated data.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Core C.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4 Core C.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

During Year 4 period of the project, we will continue with our efforts to perform hit-to-lead and lead optimization chemistry approaches on two chemical series (one main and one back-up) in each of the CHIKV and VEEV projects and on one possible chemical series for the SARS and DENV projects. Our aim is to generate at least three hit to lead compounds with reasonable ADME and in vivo PK properties that can be evaluated for potency and efficacy in a mouse model as a proof of concept study. These leads will be further optimized with the goal of identifying a preclinical candidate(s). We will also pick up re-confirmed hits from the recently completed HTS campaign against WNV and Influenza viruses to start hit to lead and lead optimization medicinal chemistry. The hits from these screens are being re-confirmed in the primary CPE based assay as well as in VTR assay and we expect to pick at least one hit for each virus to move forward to follow-up chemistry in Year 4 of the project.

Primary goals of Core C include: 1) analysis of high throughput screening data; 2) purchase and re-synthesis of promising hits for potential follow-up chemistry; 3) distribution of these compounds to the various Research Projects for evaluation in relevant assays; 4) medicinal chemistry on selected and confirmed hits (hit-to-lead and lead optimization); 5) Absorption, Distribution, Metabolism, and Excretion (ADME) and PK supports; 6) Structural biology support and; 7) Structure-based virtual screening of commercial libraries against relevant X-ray-derived models.

In continuation with the chemistry activities in Core C from Year 2, Core C performed in all of the above primary responsibilities. Compound Management Group carried out compound repository and all samples were stored in freezers before shipping to various Research Projects locations according to compounds handling and shipping methods. This group is also responsible for maintaining the Dotmatics Compound Management Database provided unique compound identification numbers (SRI Number) as well as used to store biological and ADME data. ADME and Analytical Group carried out *in vitro* ADME analysis and stored data in the Dotmatics system for retrieval and analysis by medicinal chemists. This group also carried out high resolution exact mass (HR-MS) analysis and sample purity by HPLC.

Active compounds from four HTS mass screens [Chikungunya virus (CHIKV), Venezuelan equine encephalitis virus (VEEV), West Nile Virus (WNV) and Influenza virus] which were carried out in Yr. 3, and several closely related analogs were also acquired from various commercial sources for activity re-confirmation and primary SAR- activities. All compounds were analyzed for purity by HPLC and integrity by ^{1}H NMR and HR-MS before registering in to the Dotmatics database to obtain unique identifiers and disperse to various Research Project sites for antiviral activity. Chemical synthesis work in Core C for different projects were prioritized with limited number of bench chemists based on the stages of compound development in hit-to-lead or lead optimization processes as well as availability of different antiviral assays. Medicinal chemistry approaches consist of several steps such as hit(s) identification from HTS data, hits re-confirmation, preliminary ADME properties and visual inspection of structures for uniqueness to prioritize the processes of hit selection and hit-to-lead after which analogs are then synthesized to generate SAR. A lead is then generated to further pursue lead optimization where activity and PK properties are optimized towards the identification of a candidate for evaluation in an animal efficacy model.

Research Project-1 on Flaviviruses include Dengue virus (DENV) and West Nile virus (WNV). Core C continued hit to lead chemistry efforts on three reconfirmed AD3C HTS screen hits (SRI-35847, SRI-33361 and SRI-36204) against DENV from Year 2. For initial hit to lead potentials on all three hits, a total of 50 new analogs were designed and synthesized. These compounds were submitted to Research Project-1 for antiviral activity to evaluate potency and efficacy against VEEV. These compounds were also screened in the Mirror Ball (MB) assay (SAR assay) developed by Core C for antiviral activity. A cell viability assay was also performed to evaluate cytotoxicity and *in vitro* pharmacokinetic properties of active compounds within each series were determined. Results of these studies are provided in Section G (Core Specific Information).

After the completion of the HTS screening campaign against WNV on 197K+ compounds in the third quarter of Year 3, dose response data of selected compounds was analyzed by Core C. The HTS screens were performed in the following two ways: Screen A) Targeted mechanism: Inhibition 2'-O-Methyltransferase A, and Screen B) Secondary mechanism: Direct antiviral effect. Active compounds from both screens were subjected to Pan Assay Interference Compounds (PAINS) filter followed by clustering analysis. Compounds were selected for re-confirmation assay based on their antiviral activity and cytotoxicity data, and fresh samples were acquired from

commercial sources. The samples were tested for their purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before submitting for reconfirmation assay in Research Project-1 sites. These compounds are currently being tested for their antiviral potency (EC_{90}) and efficacy (virus titer reduction assay, VTR). Results of these studies are provided in Section G (Core Specific Information).

Research Project-2 on Coronaviruses includes Severe Acute Respiratory Syndrome (SARS) virus. Hit to lead chemistry was continued from Year 2 on the hit selection from AD3C HTS screen of three compounds (SRI-35293, SRI-33684 and SRI-33911). SAR virus NanoLuc (NL) assay was developed by Assay Development Core (Core B) as the primary SAR assay to screen synthesized compounds. A total of 89 analogs of the first two hits were synthesized and screened for its potency in the NL and cytotoxicity assays to develop preliminary SAR information. The *in vitro* ADME properties of active compounds within each series were also determined. These compounds are currently being tested for their antiviral potency (EC_{90}) and efficacy (VTR) in Research Project-2 laboratory. Results of these studies are provided in Section G (Core Specific Information).

Research Project-3 on Alphaviruses includes VEE and CHIK viruses. Core C continued chemistry from Year 2 on a MLPCN re-confirmed hit SRI-33394, which showed excellent antiviral activity in a Normal Human Dermal Fibroblasts (NHDF) cell line against VEEV. Approximately 40 analogs were designed, synthesized and submitted to Research Project-3 to test antiviral activity in Year 3. On active compounds from this series, ADME properties, such as human and mouse microsomal stability, aqueous solubility and logD were also determined on active compounds. Results of these studies are provided in Section G (Core Specific Information).

After the completion of VEEV HTS screening campaign on 197K+ compounds in the first quarter of Yr. 3, dose response data was analyzed by Core C. Active compounds were subjected to PAINS filter to triage unwanted molecules followed by clustering analysis. Compounds were selected for the re-confirmation assay based on their antiviral activity and cytotoxicity data, and fresh were acquired from commercial sources. These compounds were tested for purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before submitting to the reconfirmation assay in Research Project-3 laboratory. The compounds were tested for antiviral potency (EC_{90}) and efficacy (VTR). ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, were evaluated on all confirmed hits before initiating chemistry on two of the best lead compounds. Results of these studies are provided in Section G (Core Specific Information).

Core C also continued with medicinal chemistry efforts in CHIKV from Year 2. This is one of the most advanced programs among all of the research projects. Medicinal chemistry efforts were initiated by developing hit SRI-33366 to lead SRI-34963. Lead optimization of SRI-34963 was pursued with a goal of identifying a compound with improved ADME properties such as mouse microsomal stability and solubility (>10 μM) while retaining its antiviral potency and efficacy to test in a mouse model. Approximately 120 analogs were synthesized and tested for antiviral potency and efficacy using the NHDF (normal human dermal fibroblasts) cell line in Research Project 3 laboratory. Several of the compounds showed excellent antiviral potency and efficacy with reasonable ADME properties. After careful evaluation of activity and ADME profiles of potential lead compounds, five leads were selected for *in vivo* pharmacokinetic (PK) studies.. These efforts also led to the identification of a second generation lead molecule SRI-36498 with improved PK and antiviral properties which will be evaluated in a mouse model for its potency and efficacy. The Structural Biology Group has also performed studies towards the target identification of SRI-34963, the parent lead, in the CHIKV virus based on preliminary data generated from virus resistant studies by Research Project 3 labs. Results of these studies are provided in Section G (Core Specific Information).

After the completion of HTS screening campaign on 197K+ compounds against CHIKV in first quarter of Year 3, dose response data was analyzed by Core C. The hits were analyzed by clustering analysis and PAINS filtration. The data on these compounds were sorted by EC_{50} values and selectivity index (SI). Some compounds were also filtered by visual inspection of structures possessing unwanted functional groups, core structure uniqueness and commercial availability. Compounds were selected and repurchased for re-confirmation in antiviral assay. The fresh samples were tested for their purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before submitting to the reconfirmation assay in Research Project-3 laboratory. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, were evaluated on all confirmed hits before initiating chemistry on two of the best lead compounds. Results of these studies are provided in Section G (Core Specific Information).

In this project there was also an attempt to identify some cross virus active molecules within the virus family. The reconfirmed hits from VEEV and CHIKV hits were cross tested which resulted in two compounds showing potential activity against each virus. A total of 24 new analogs have been synthesized recently and submitted to Core B to screen in a combination assay of antiviral effect (EC_{50} and VTR) as well to Research Project-3. Results of these studies are provided in Section G (Core Specific Information).

Research Project-4 on Influenza viruses includes three strains (H1N1, H3N2 and H5N1). In continuation with Core C chemistry efforts from Year 2 on SRI-34993 with antiviral activity in two of the strains (H1N1 and H3N2), 25 commercial analogs were purchased and screened against both virus types in MDCK cells. While waiting on the HTS mass screen for influenza to be completed, Core C collected a set of 32 potent reconfirmed HTS hits from SARS, VEEV, CHIKV and DENV screens and were supplied these compounds to Research Project-4 to test for antiviral activity against H3N2 strain using HEK293 and MDCK cells. After completion of the HTS screening campaign in the third quarter of Year 3, dose response data using HEK293 cells was analyzed by Core C. Hits were subjected to PAINS filtration and clustering analysis. The PAINS filtered hits were then screened for antiviral activity against H1N1 and H3N2 strains using MDCK cells at two concentrations. Compounds that were active in both strains and possessed low cytotoxicity were selected. A fresh sample is now being acquired which will be tested for purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before submitting for evaluation of antiviral activity in CPE and VTR assay. Results of these studies are provided in Section G (Core Specific Information).

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

We have instituted an undergraduate internship program with University of Alabama at Birmingham Chemistry Department. One undergraduate student worked in Core C and synthesized compounds for the CHIKV program.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Research Material	Limited quantities of most synthetic compounds will be made available to qualified individuals for research purposes once the pertinent data has been published. As stated in the original Resource Sharing Plan for Core C, once published, and while compound supplies last, we will make research samples of synthetic compounds available to the scientific community for use as chemical probes and for other biological studies. Moreover, we fully expect all biological and chemical data to be published in scientific manuscripts after appropriate patent protection is in place. At this early stage of chemistry, there is no specific resource sharing to report.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

Core C was designed to escalate activities in Years 3 and 4 in order to accommodate hit-to-lead phases in all four projects. However, the activities of Core C is dependent of Core B finishing the HTS screens and implementation of SAR assays. Core C successfully initiated hit confirmation and hit to lead chemistry from HTS mass screen data finished in Year 2. However, HTS screens on WNV and Influenza were completed in second quarter of Year 3 and activities of Core C on these specific projects are dependent upon the completion of the high throughput screens for these viruses. We have recently analyzed HTS data on WNV screen and prioritized chemical series of interest. Fresh commercial samples have been ordered and analyzed for their purity and integrity. Most of the hits were submitted to the biology laboratory for reconfirmation. As we receive the data, we will prioritize which hits to initiate medicinal chemistry. Similarly, the Influenza project HTS hits reconfirmation was recently completed using MDCK cells and the data is being analyzed. Medicinal chemistry efforts will soon start in Year 4 on the hits from these two screens in addition to ongoing efforts on six hit to lead compounds and two lead optimization compounds for other viruses.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-8280

RESEARCH & RELATED BUDGET - SECTION A & B **FINAL**

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Ashish		Pathak		Project Leader	(b)(4); (b)(6)				31,384.00	14,624.00	46,008.00
2.	Dr	Corinne		Augelli-Szafran		Co-Project Leader					16,463.00	7,672.00	24,135.00
3.	Dr	Mark		Suto		Co-Project Leader					16,463.00	7,672.00	24,135.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **94,278.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			43,416.00	20,232.00	63,648.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
13	9 Chemist, 2 Scientist, 1 PK Tech., 1 Project Mgr.				386,553.00	180,134.00	566,687.00
14	**Total Number Other Personnel**					**Total Other Personnel**	**630,335.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**724,613.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	292,288.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Hazardous Waste	3,250.00
Total Other Direct Costs	**295,538.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,025,151.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH - Salaries & Benefits	120.0	724,613.00	869,535.00
2. G & A - Total Direct Cost + OH	20.0	1,894,686.00	378,937.00
3. CFC - Salaries & Benefits	7.3	724,613.00	52,897.00
4. CFC - Total Direct Cost + OH	1.0	1,894,686.00	1,895.00
		Total Indirect Costs	**1,303,264.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**2,328,415.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: SR 000502793-011 Core C Yr 4 Just.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification — Medicinal Chemistry and Lead Development Core

Ashish K. Pathak, Ph.D. (Advanced Research Scientist, Chemistry Department) will serve as a Core Leader of the Medicinal Chemistry and Lead Development Core. He has over 26 years of experience in medicinal and synthetic organic chemistry, including significant experience in anti-infective and vaccine adjuvant drug design. He has established himself as an independent researcher at Southern Research (SR) and previously led research groups in the Department of Chemistry at Western Illinois University as an Assistant Professor before joining SR. Currently, he manages the high-throughput parallel synthesis group and is involved in several internal drug discovery projects as a supervisor in medicinal chemistry. He has extensive experience in all aspects of a medicinal chemistry program, which extends across the spectrum of early lead discovery to lead optimization. He has been PI of two R21 NIH-funded programs in the area of viral vaccine adjuvant discovery. In collaboration with the PIs and other Co-Investigators in the Center, he will oversee all aspects of the medicinal chemistry effort, including hit triage and synthetic target selection, target design, synthesis, and the planning of synthetic routes, compound characterization, structure-activity relationship analysis and interpretation of biological results, cheminformatics and molecular modeling, report, manuscript, and patent application preparation and overall project management of lead optimization chemistry. Dr. Pathak will devote (b)(4) months to the Core during Year 4.

Mark J. Suto, Ph.D. (Vice President, Drug Discovery Division) and Corinne E. Augelli-Szafran, Ph.D. (Director, Chemistry Department) will serve as Co-Core Leaders of the medicinal chemistry core for the proposed project. Each has approximately 30 years of drug discovery experience across multiple therapeutic areas, including all steps in discovery and development, with a focus on discovering, developing, and advancing new compounds to the clinic in an effective and efficient manner, while ensuring that all of the needed data for regulatory filings are properly gathered and maintained. In addition to experience in early lead identification and discovery, target validation, and lead optimization, Dr. Suto has served on clinical development teams and managed the preparation of drug product for clinical trials. For the current project, Drs. Pathak, Suto, and Augelli-Szafran will work as a team in the selection of hit compounds to move forward, and in the subsequent selection of lead candidates. They will be involved in all aspects of the medicinal chemistry program, including the design of optimal development strategies for individual lead candidates. Dr. Suto will devote (b)(4) months and Dr. Augelli-Szafran will devote (b)(4) months to the Medicinal Chemistry and Lead Development Core in Year 4.

Wei Zhang, Ph.D. (Research Scientist, Chemistry Department) has a broad background in development and application of computational methods for the modeling and understanding of biological systems. He holds a Ph.D. in computational chemistry and has had postdoctoral training in structural biology and computer-aided drug design. For this project, Dr. Zhang will provide computational chemistry support for each hit follow-up and lead optimization project, including cheminformatics, clustering analyses, and hit triage filtering, calculation of molecular properties, structure activity relationship (SAR) analysis and model building (such as pharmacophore and homology as appropriate to the given project), searches for commercial analogs of hit compounds, and virtual screening against selected viral target proteins. He will also assist in the preparation of appropriate reports, manuscripts, and patent applications. Dr. Zhang will devote variable effort as needed, depending upon the number of active projects, beginning with (b)(4) months in Year 4.

Mousheng (Mason) Wu, Ph.D. (Structural biologist, Chemistry Department) has extensive training and experience in protein sciences and structural biology including protein expression, protein purification, functional characterization, protein identification and protein structure determination. He holds a Masters of Science degree in Biochemistry and a Ph.D. degree in X-ray crystallography. His lab currently focuses on structure-based drug discovery by providing the details of protein-compound atomic structure to develop new compounds. For this project, Dr. Wu will provide variable efforts once the viral proteins which the anti-viral compounds are targeting are identified. His laboratory will provide support such as cloning, expression and purification of target proteins, characterization of protein-compound binding, and determination of protein-compound structures. The goal of his laboratory in this project is to determine the atomic structure of protein-compound complexes and provide the details to the chemists to assist with the design of more potent compounds. Dr. Wu will devote his efforts for different projects, beginning with (b)(4) months in Year 4.

Kaleem Ahmed, Ph.D., Nikhil Madadi, Ph.D, Valerie Smith, Ph.D., Theresa Nguyen, Ph.D., and Han-Xun Wei, Ph.D. (Chemists, Department of Chemistry), will provide chemistry services. They will be directly responsible for day-to-day synthetic activities in the laboratory. They will perform compound synthesis, compound scale-up, and literature searching, and will assist in target selection, analog design, synthetic methodology, compound characterization, data analyses, cheminformatics and molecular modeling. Each of these chemists has several years of post-doctoral research experience in the design and preparation of several different types of compounds and in the analysis of biological data for structure-activity relationships. Each will devote (b)(4) effort to this project over the duration of the grant award in performing hit to lead chemistry and lead optimization on active compounds/series against various viruses (Projects 1-4) and in the scaleup synthesis of lead molecules for animal studies. In Grant Year 4, all 5 chemists will devote (b)(4) months.

Omar Moukha-Chafiq, Ph.D. (Research Chemist, Chemistry Department) will support Dr. Pathak in running the chemistry core. He has more than 14 years of extensive research experience in the synthesis of potential anticancer, antiviral, antibiotic nucleoside chemistry and small-molecule drug discovery. Because of his broad synthetic background and his experience with solid-, solution-, and liquid- phase methodologies, employing both robotic and manual protocols, he has been the lead chemist on several NIH-funded grant projects. In Grant Year 4, Dr. Moukha-Chafiq will devote (b)(4) months of his time on this project. Mr. Sam Tanner, M.S., a Chemist in Dr. Omar Moukha-Chafiq's group, will also devote (b)(4) months in Year 4 in hit to lead chemistry as well as in large scale synthesis as needed.

David Poon (Chemist, Supervisor of Compound Management, Chemistry Department) has extensive experience in managing in-house synthesized compounds and commercial libraries. He will provide integrated informatics support, including compound tracking, data capture, and data storage, backup, and retrieval. He is in charge of maintaining our in-house Dotmatics registration database, which is used extensively in this program to assign a unique identifier to each compound synthesized or commercially-acquired. This identifier number is used throughout the Center to track compounds and any associated data. He is also responsible for sample preparation and distribution to different project teams and to Core B. He will devote (b)(4) months to this program in Year 4.

(b)(6); (b)(3):7 U.S.C. § 8401 M.S., PMP (Divisional Project Manager, Drug Discovery Division), has five years of experience in coordinating and managing research projects in the Drug Discovery Division at SR. She will work with Dr. Suto and the other project leaders to ensure a timely and efficient delivery of Core services to the overall program and will devote (b)(4) months in Year 4 to the program.

In Grant Year 4 (and variable in subsequent years), we have allocated ~$34,000 for the purchase of commercial analogs of hit compounds in order to generate preliminary SAR data. This estimate is based on at 6 scaffolds for follow-up analoging, and 50 commercial analogs being purchased (10-20 mg quantities) for each scaffold at approximately $100/compound.

Each of the synthetic chemists has been allocated a reagent budget of ~$36,000 per year per FTE, an estimate based on our previous experience in lead optimization chemistry programs. This budget covers starting materials, specialized reagents, solvents, chromatography supplies, resins and solid-phase synthesis supports, glassware, plastic ware, and other disposables as well as spectroscopy and compound characterization expenses. The chemistry supply cost for synthesis is proposed to be $200,000 for Grant Year 4.

Approximately $3,250 is allocated for hazardous waste disposal for Grant Year 4.

Donghui Bao, Ph.D. (Research Scientist, Chemistry Department) is the supervisor of the bioanalytical drug discovery laboratory in the Chemistry Department at SR. He has extensive experience in developing and validating efficient bioanalytical methods for quantitative analysis of novel pharmaceuticals, metabolites, and endogenous compounds for use in clinical and non-clinical research and he has a working knowledge of GLP regulations. He also has expertise in quantitative bioanalytical validation, including solid-phase extraction

(SPE), high-performance liquid chromatography (HPLC), and mass spectrometry (MS)/MS development and optimization, operation, maintenance, and calibration of liquid chromatography (LC)-MS/MS instrumentation and Rapid Trace SPE instrumentation, research involving animal models including oral, intravenous, and intramuscular dosing, aseptic cell culture techniques, including the use of cultured and freshly isolated hepatocytes, and the handling and analysis of radioisotopes by Liquid Scintillation and Gamma Counting (^{3}H, ^{14}C, ^{51}Cr, ^{125}I). Dr. Bao will be responsible for overseeing PK/ADME studies in all lead optimization projects. He will devote (b)(4) months to this project. Dr. Robert Deimler, Ph.D. (Associate Research Chemist, Chemistry Department) will assist Dr. Bao in day-to-day activities to carry out experimental analytical work in his lab. Dr. Deimler will also perform high resolution mass spectral studies on all final compounds synthesized in this program. Dr. Deimler will devote (b)(4) months in Year 4 to this project.

One technician (TBD, 4.2 calendar months) for animal work will devote 40 hrs/PK study/compound for pharmacokinetic and toxicological profiling of potential drug candidates emerging from the lead optimization program under the direction of Dr. Bao. $58,021 annually has been allocated to cover PK/ADME supplies and animal costs for these studies.

$5,000 annually is allocated for the Core Leader and Co-Core Leaders to attend the required NIAID CETR Program Meeting. This will total $15,000 in Year 4 for travel funds, which will support meeting registration, abstract submission fees, round-trip airfare, ground transportation, hotel accommodation, and meals for this event.

Note: ICD rate for CFC Total DIrect Cost + OH is 0.10%; online form does not allow less than 1%